CELL PLASTICITY IN CANCER:
CUES FROM VIRUS-HOST INTERACTIONS

Pirita Pekkonen

Research Programs Unit
Translational Cancer Biology
Faculty of Medicine

and

Doctoral Programme in Biomedicine (DPBM)
University of Helsinki
Finland

ACADEMIC DISSERTATION

To be publically discussed with the permission of the Faculty of Medicine,
University of Helsinki, in Auditorium 3, Biomedicum Helsinki,
Haartmaninkatu 8, on August 14th 2015, at 12 o'clock

Helsinki 2015

SUPERVISOR

Päivi Ojala

Ph.D., K. Albin Johansson Research Professor
Translational Cancer Biology Program
Research Programs Unit
Biomedicum Helsinki
University of Helsinki
Finland

REVIEWERS

Sirpa Jalkanen

M.D. Ph.D., Professor of Immunology
Medicity Research Laboratory
University of Turku
Finland

and

Eeva Auvinen

Ph.D., Docent in Virology
Department of Virology
Haartman Institute
University of Helsinki
Finland

OPPONENT

Enrique A. Mesri

Ph.D., Associate Professor of Microbiology & Immunology
Sylvester Comprehensive Cancer Center
University of Miami Health System
Miami, Florida, USA

ISBN 978-951-51-1420-4 (paperback)
ISBN 978-951-51-1421-1 (pdf)
http://ethesis.helsinki.fi
Unigrafia Oy
Helsinki 2015

To my loved ones

CONTENTS

ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals.

I. **Pekkonen P.**, Järviluoma A., Zinovkina N., Cvrljevic A., Prakash S., Westermarck J., Evan G.I., Cesarman E., Verschuren E.W., and Ojala P.M.: KSHV viral cyclin interferes with T-cell development and induces lymphoma through Cdk6 and Notch activation *in vivo*. *Cell Cycle.* 2014;13(23):3670-84.

II. Buss H.*, Handschick K.*, Jurrmann N.*, **Pekkonen P.*,** Beuerlein K., Müller H., Wait R., Saklatvala J., Ojala P.M., Schmitz M.L., Naumann M., Kracht M. Cyclin-dependent kinase 6 phosphorylates NF-κB p65 at serine 536 and contributes to the regulation of inflammatory gene expression. *PLoS One.* 2012;7(12):e51847. *equal contribution.

III. Cheng F.*, **Pekkonen P.*** Laurinavicius S.*, Sugiyama N., Henderson S., Günther T., Rantanen V., Kaivanto E., Aavikko M., Sarek G., Hautaniemi S., Biberfeld P., Aaltonen L., Grundhoff A., Boshoff C., Alitalo K., Lehti K., Ojala P.M.: KSHV-initiated Notch activation leads to membrane-type-1 matrix metalloproteinase-dependent lymphatic endothelial-to-mesenchymal transition. *Cell Host Microbe*. 2011 Dec 15;10(6):577-90. *equal contribution.

IV. **Pekkonen P.**, Balistreri G., Tatti O., Taiwo A, Perälä N., Zinovkina N., Niiranen O., Repo, P., Icay K., Hautaniemi S., Lehti K., Ojala P.M.: Tumor cell interaction with lymphatics contributes to melanoma progression. *Manuscript*.

Additional unpublished material is also presented.

Publication III was also used in the thesis of M.D. Ph.D. Fang Cheng.

ABBREVIATIONS

3D	three-dimensional
ADAM	a disintegrin and metalloproteinase
AIDS	acquired immunodeficiency syndrome
AKT	protein kinase B
ANG	angiopoietin
AP	activator protein 1
ATG3	autophagy related 3
α-SMA	alpha smooth muscle actin
BEC	blood endothelial cell
BMP	bone morphogenetic protein
CAF	cancer associated fibroblast
CCL/R	CC chemokine ligand/receptor
CDC	Cell division control protein
CXCL/R	CXC chemokine ligand/receptor
CD	cluster of differentiation
CDK	cyclin-dependent kinase
COUP-TFII	COUP transcription factor 2
COX-2	prostaglandin-endoperoxide synthase 2
CREB	cyclic AMP response element-binding protein
DAPT	N-[N-(3,5-Difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester
DDR	DNA damage response
DED	dead effector domain
DLL4	delta like ligand 4
DNA	deoxyribonucleic acid
dsDNA	double stranded DNA
EBV	Epstein-Barr virus
EC	endothelial cell
E-cadherin	epithelial cadherin
ECM	extracellular matrix
EMT	epithelial to mesenchymal transition
EndMT	endothelial to mesenchymal transition
ErbB2	receptor tyrosine-protein kinase ErbB2, also known as HER2
ERK	extracellular signal-regulated kinase
FACS	fluorescence-activated cell sorting
FAP	fibroblast activating protein
FGF	fibroblast growth factor
FSP-1	fibroblast specific protein 1
GFP	green fluorescent protein
HAART	highly active antiretroviral therapy
H2Kb	H-2 Class I Histocompatibility Antigen K-B
HES1	hairy enhancer of split 1
HEY1	hairy/enhancer-of-split related with YRPW motif protein 1
HGF	hepatocyte growth factor
HHV-8	human herpes virus 8
HIF-1α	hypoxia inducible factor 1α
HIV	human immunodeficiency virus
HPV	human papillomavirus
HUVEC	human umbilical endothelial cell
ICAM-1	intercellular adhesion molecule 1
IGF-1	Insulin-like growth factor 1
IκB	NF-κB light polypeptide gene enhancer in B-cells inhibitor
IKK	IκB kinase
IL	interleukin
iPSC	induced pluripotent stem cells
KIT	Mast/stem cell growth factor receptor (SCFR)
K-LEC	KSHV infected lymphatic endothelial cell

KS	Kaposi's sarcoma
KSHV	Kaposi's sarcoma herpesvirus
LANA	latency associated nuclear antigen
LC3	Microtubule-associated protein 1A/1B-light chain 3
LEC	lymphatic endothelial cell
LYVE-1	lymphatic vessel endothelial hyaluronan receptor 1
MAPK	mitogen activated protein kinase
miRNA	microRNA
MMP	matrix metalloproteinase
MOI	multiplicity of infection
mRNA	messenger RNA
MR1	mannose receptor 1
MT-MMP	membrane type matrix metalloprotease
mTOR	mammalian target of rapamycin
NF1	neurofibromin 1
NFAT	Nuclear factor of activated T-cells
NF-κB	nuclear factor kappa-B
NICD	Notch intracellular domain
NOD/SCID	nonobese diabetic/severe combined immunodeficient
NPM	nucleophosmin
p21	cyclin-dependent kinase inhibitor 1
p27	cyclin-dependent kinase inhibitor 1B
p65	protein 65
PAGE	polyacrylamide gel electrophoresis
PAR-1	Proteinase-Activated Receptor 1
PCR	polymerase chain reaction
PDGF	platelet-derived growth factor
PECAM-1	platelet-endothelial cell adhesion molecule 1
PEL	primary effusion lymphoma
PI3K	Phosphatidylinositol-4,5-bisphosphate 3-kinase
PTEN	Phosphatase and tensin homolog
Prox-1	prospero homeobox 1
RAS	'Rat sarcoma' protein
Rb	retinoblastoma protein
RBP-Jk	Recombining binding protein suppressor of hairless
RNA	ribonucleic acid
shRNA	short hairpin RNA
siRNA	short interfering RNA
SMAD	Mothers against decapentaplegic homolog
SOX18	SRY (sex determining region Y)-box 18
STAT	signal transducers and activators of transcription
T-ALL	T-cell acute lymphoblastic leukemia
TIMP	tissue inhibitors of metalloproteinases
TAM	tumor associated macrophage
TGF-β	transforming growth factor-β
TNF	tumor necrosis factor
TP53	tumor protein 53
TPA	12-O-tetradecanoyl phorbyl-13-acetate
TSA	tyramide signal amplification
UV	ultraviolet
VCAM-1	vascular adhesion molecule 1
v-cyclin	viral cyclin
VE-cadherin	vascular endothelial cadherin
VEGF	vascular endothelial growth factor
vFLIP	viral FLICE-inhibitory protein
vGPCR	viral G protein-coupled receptor
ZEB1/2	zinc finger E-box binding homeobox 1/2
ZO-1	Zonula occludens, tight junction protein 1

ABSTRACT

Human tumorigenesis is a process in which a normal cell needs to acquire multiple characteristics to become malignant and metastatic. In short, these so called cancer hallmarks include increased proliferation and cell survival, as well as the ability to invade into the surroundings, induce angiogenesis, and finally metastasize to distant sites. These traits are regulated in a variety of different ways. However, some embryonic signaling pathways, including the Notch pathway, are able to regulate many of these processes. Furthermore, it has been shown that these signaling pathways can be deregulated in cancer, and that their untimely activation can lead to malignancies. In this study, Kaposi's sarcoma herpesvirus (KSHV) associated malignancies, namely Kaposi's sarcoma (KS) and primary effusion lymphoma (PEL), as well as melanoma have been used as model cancers. In all these malignancies, the tumor cells show alterations in cell identity and lineage marker expression, i.e. signs of cellular de- or transdifferentiation. In addition, the Notch pathway has been shown to be overly active in all of them. Thus, this thesis has focused on how the pro-tumorigenic traits are affected by cell plasticity and reprogramming in these cancers, and how the signaling pathways leading to these phenotypes, most notably Notch, are in turn regulated. Firstly, the results show that *in vivo* expression of a KSHV oncogene, viral (v-)cyclin, leads to activation of Notch signaling through Notch3 upregulation as well as fine-tuning of the NF-κB pathway through Cdk6 mediated phosphorylation. These changes in turn lead to defects in T-lymphocyte differentiation and immune functions, as well as to the development of T-cell lymphomas. Secondly, this work demonstrates that KSHV infection in primary lymphatic endothelial cells (LECs) in three dimensional (3D) cell culture model leads to activation of a morphogenic process, endothelial to mesenchymal transition (EndMT), and increased invasiveness through activation of the Notch pathway and matrix metalloproteinase MT1-MMP. Lastly, the data show that the changes in cell plasticity contributing to tumorigenic traits are not confined to virally induced cancers. Melanoma cell interaction with LECs leads to activation of the Notch pathway and increased adhesive, invasive, and metastatic properties of the tumor cells. In conclusion, the results show that regulation of cell plasticity through the Notch pathway takes place in different types of cancers, and it can affect several steps of tumorigenesis. A thorough and comprehensive understanding of the processes discovered herein may help develop better and more efficient treatments for these largely fatal malignancies.

TIIVISTELMÄ

Monien normaalin solun ominaisuuksien tulee muuttua, ennen kuin solu muuntuu syöpäsoluksi ja pystyy leviämään elimistössä. Solun täytyy muun muassa pystyä jakautumaan hallitsemattomasti, tunkeutumaan ympäristöönsä, erittämään veri- ja imusuonien kasvuun vaikuttavia tekijöitä sekä lopulta pystyä hyödyntämään veri- ja imusuonistoa levitäkseen ympäriä kehoa. Syöpäsolujen eri ominaisuuksia säätelevät tyypillisesti eri signalointireitit, mutta eräät sikiönkehityksen aikana aktiivisesti toimivat reitit, kuten Notch-signalointi, voivat vaikuttaa moniin syöpäsolun ominaisuuksiin. Tällaisten signalointireittien yliaktiivisuus onkin liitetty syövän syntyyn ja leviämiseen. Tässä tutkimuksessa on käytetty malleina Kaposin sarkoomaan liittyvän herpesviruksen (KSHV) aiheuttamia syöpiä, Kaposin sarkoomaa (KS) ja primaaria efuusiolymfoomaa (PEL), sekä melanoomaa. Näissä kaikissa syövissä on havaittavissa, että syöpäsolujen soluidentiteetti on heterogeeninen, ja että syöpäsolut pystyvät ohjelmoitumaan uudelleen kasvuolosuhteidensa mukaan. Näille syöville on yhteistä myös, että Notch-signalointireitti on aktivoitunut. Tutkimukseni aiheena oli solujen muovautuvuus- ja ohjelmoitumiskyvyn vaikutukset syövän syntyyn ja leviämiseen ja näiden prosessien säätely. Työni ensimmäisessä osassa osoitin, kuinka KSHV:n onkogeenin v-sykliinin ilmentyminen hiiressä johtaa Notch- ja NF-κB- signalointireittien aktivoitumiseen ja sitä kautta T-solujen erilaistumisen ja toiminnan häiriöön sekä T-solulymfooman kehittymiseen. Seuraavaksi näytin, kuinka KSHV-infektio imusuonten seinämän soluissa johtaa Notch-signaloinnin aktivoitumiseen ja solujen uudelleenohjelmoitumiseen mesenkyymisolujen kaltaisiksi, jolloin ne pystyvät tehokkaammin tunkeutumaan ympäristöönsä. Lopuksi osoitin, että edellä kuvatut mekanismit eivät ole aktiivisia ainoastaan syöpävirusten aiheuttamissa kasvaimissa, vaan että myös melanoomasolujen interaktio imusuonten solujen kanssa aktivoi Notch-signalointia ja johtaa syöpäsolujen lisääntyneeseen adheesio-, invaasio- ja metastasoimiskykyyn. Notch-signalointi ja syöpäsolujen muovautumis- ja uudelleenohjelmoitumiskyky säätelevät siis monentyyppisten syöpien kehittymistä ja leviämistä. Näiden prosessien perusteellinen tuntemus mahdollistaa parempien ja tehokkaampien hoitojen kehittämisen näitä huonoennusteisia syöpiä vastaan.

INTRODUCTION

The human tumor viruses, human papillomavirus (HPV), hepatitis B and C viruses, Epstein Barr virus (EBV), Kaposi's sarcoma herpesvirus (KSHV), human T-cell lymphothropic virus, and Merkel cell polyomavirus, are estimated to cause 15-20% of all cancers worldwide. During their evolution they have hijacked cellular genes to their own genome which to date share homology which their host. KSHV genome consists of more than hundred open reading frames, including homologs of cyclin D, Flice inhibitory protein (FLIP) and G-protein coupled receptor, and it has proven to be an important tool in cancer biology research in general. An examination of virus induced tumors, and comparison of them to the human systems has potential to provide insight into the mechanisms and functions of both.

Human tumorigenesis is a cascade of consecutive or simultaneous events that lead to the formation of a malignant cancer cell that can gain the ability to spread in the body. This process usually takes decades, and thus it is not surprising that all cancer cells within the same tumor are not identical, but exhibit signs of cellular heterogeneity. Moreover, it has been shown that deregulation and untimely activation of differentiation processes can alter the fate of the cancer cell: for example, expression of oncogenic c-Myc can lead to both altered differentiation and initiation of the tumorigenic process, whereas epithelial to mesenchymal transition has been shown to be crucial for the invasive capacities of epithelial tumors. However, the interplay of differentiation and tumorigenesis and the different phases of the process have remained rather obscure in many tumor types. Tumor cells in KSHV associated malignancies, Kaposi's sarcoma (KS) and primary effusion lymphoma (PEL), show signs of widespread lineage marker expression, suggesting that deregulation of cellular differentiation pathways and cell plasticity could contribute to the tumorigenesis of these cancers. Malignant melanoma shares similar features with KS and PEL, including plastic nature of the tumor cells associated to Notch pathway activation. Thus, we hypothesized that similar mechanisms might be involved in controlling its progression as well.

REVIEW OF THE LITERATURE

1. HUMAN TUMORIGENESIS

All mammals have developed similar molecular mechanisms to control cell growth, differentiation and death. If the balance between these processes is disturbed, the cells can start proliferating uncontrollably. This might eventually lead to formation of a malignant tumor, cancer. The transformation from a normal cell to a cancer cell is a multistep process occurring via various routes in different malignancies. However, the required features are similar in all cancers (reviewed in Figure 1A, and (Hanahan and Weinberg, 2000)). Briefly, transformed cells are autonomous from growth signals and resist inhibitory growth signals by inactivating the growth suppressive pathways and by upregulating cell survival signaling. They acquire limitless replicative potential by regaining the expression of an enzyme that prolongs the telomeres. The cancer cells are able to evade programmed cell death, apoptosis, by deregulating the apoptotic signaling pathways. To grow beyond certain size limit, the tumors regulate their oxygen and nutrient supply by inducing angiogenesis. Finally, in order to spread further, the tumor cells can develop capabilities which allow tissue invasion and metastasis to distant organs (Hanahan and Weinberg, 2000). In recent years, it has also become increasingly clear that cancer cells cannot gain all these abilities by themselves, but the surrounding stromal cells and the extracellular matrix (ECM) function actively in the transformation process in collaboration with the tumor cells (Quail and Joyce, 2013).

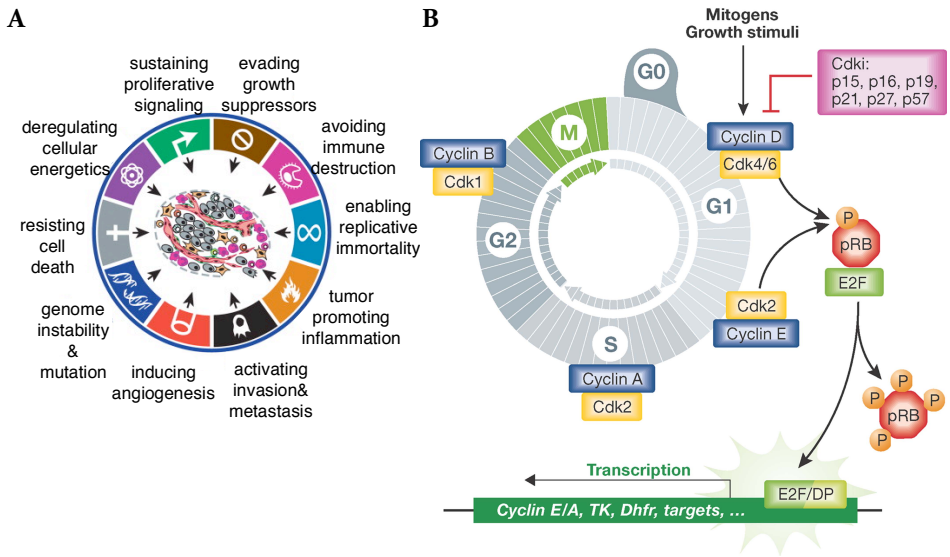

Figure 1. Cancer hallmarks (A) and cell cycle progression (B).

(modified from (A) (Hanahan and Weinberg, 2011) and (B) (Aguilar and Fajas, 2010))

1.1 CELL TRANSFORMATION

1.1.1 Cell cycle and proliferation signals

For a cell to proliferate, the cell needs go through a cell division process called cell cycle. The main phases of the cell cycle (summarized in Figure 1B and ref. (Malumbres and Barbacid, 2009)) are DNA replication (S) phase and segregation of the newly synthesized daughter chromosomes in mitosis (M phase). These phases are preceded by two gap phases (G1 and G2), in which the cell is preparing for the S and M phases. Fluctuating levels of proteins specific for their respective cell cycle phase, called cyclins, control the phases. The cyclins act together with specific kinases, cyclin dependent kinases (CDKs). In the G1 phase, the cyclin D – CDK4/6 complex is active, which leads to phosphorylation of the retinoblastoma protein (pRb), its release from the transcription factor E2F, and synthesis of the G1-S proteins. These include cyclin E, which is an S phase cyclin that interacts with CDK2. In the early G2 phase, CDK2 interacts with cyclin A2, which in turn activates CDK1 later in the G2 phase. As a result, the cell enters mitosis, which is driven by the cyclin B-CDK1 complex. The cell cycle is further controlled by cell cycle inhibitors, which function by binding to and preventing the function of the cyclin-CDK complexes. The inhibitors are divided into two classes depending on their substrates. The INK4 family inhibitors block the cyclin D-CDK4/6 complex, whereas the CIP/KIP family members p21, p27, and p57 mainly regulate the cyclin E-CDK2 complex. Genetic mouse studies have revealed that apart from some highly differentiated cells, the interphase CDKs (CDK2, 4, 6) and cyclins (cyclin Ds and E) are dispensable for executing the cell cycle during development (Santamaria et al., 2007). Thus, it seems that CDK1 is sufficient to drive the cell cycle in normal conditions in mammals, and that the other cyclins are needed for additional control of proliferation of a wide array of specialized cells (Malumbres and Barbacid, 2009).

The cell cycle is usually tightly regulated by mitogenic and anti-mitogenic signals. Typical signaling leading to cell proliferation involves a cell-extrinsic growth signal, which is mediated by binding of a ligand to a cell surface tyrosine kinase receptor. This initiates a phosphorylation cascade of various intracellular downstream targets leading to gene expression via activated transcription factors and finally cell cycle progression. Normal cells cannot proliferate without extracellular signals, which can be secreted growth factors, extracellular matrix (ECM) components, or intercellular interaction molecules such as integrins (Hanahan and Weinberg, 2000). However, in cancer, the cell cycle machinery might be activated without appropriate upstream regulation, in a process called unscheduled proliferation (Malumbres and Barbacid, 2009). Autonomous activation of cell cycle progression can occur at all steps of the proliferation cascade, including the cell cycle itself. In mice, transgenic expression of cyclin D/E, as well as germline silencing of p21 or p27 coding genes, gives rise to a variety of cancers (Santamaria and Ortega, 2006). pRb again functions as a haploinsufficient tumor suppressor, and pRb+/- mice develop endocrine tumors (Malumbres and Barbacid, 2001). Active cyclin D1- CDK4 complexes have also been shown to be necessary for breast cancer development due to ErbB2 oncogene expression (Landis et al., 2006, Yu et al., 2001, Yu et al., 2006), suggesting that CDK4/6 activity is needed in cancer cells in contrast to most normal cells. This is

supported by reports showing that D- or E-type cyclins are often upregulated in cancer, whereas cell cycle inhibitors and pRb genes are frequently silenced, both leading to hyper activation of *CDK4*/*6* seen in multiple tumor types including lymphoma, sarcoma, and melanoma (Malumbres and Barbacid, 2009).

1.1.2 Regulation of apoptosis

The programmed cell death, apoptosis, is utilized by multicellular organisms to balance the cell number and dispose of unnecessary or damaged cells. The apoptotic machinery can be activated by extracellular stimuli such as tumor necrosis factor alpha (TNFα), or intracellular stress stimuli, including DNA damage and overly active proliferation signals (Adams and Cory, 2007). The stress stimuli activate pro-apoptotic BH3 domain containing proteins, which are balanced by the anti-apoptotic Bcl-2 like proteins. The pro-apoptotic Bcl-2 family members function more downstream of the apoptotic cascade to control the permeability of the mitochondrial membrane to release cytochrome C (Adams and Cory, 2007). Extra- and intracellular signals converge at the activation of apoptosis effector proteases called caspases, which in turn cleave hundreds of cellular proteins and DNA (Adams, 2003). Finally, the apoptotic cascade leads to disruption of cellular membranes, destruction of the cell skeleton, extrusion of the cytosol, degradation of chromosomes, and fragmentation of nuclei, which are then engulfed by the neighboring cells as well as the phagocytic system.

In normal cells, many of the intracellular signals leading to apoptosis, most notably DNA damage but also hypoxia and oncogene hyperexpression, are sensed by the activation of p53. p53 is a transcription factor which promotes apoptosis by inducing the expression of pro-apoptotic Bcl-2 family members Noxa and Puma. In addition to apoptosis, p53 activation can counteract the cell proliferation of damaged cells by activating the cell cycle checkpoints and inducing senescence (Brady and Attardi, 2010). As oncogene activation in a normal cell would lead to initiation of apoptosis, there is a selection pressure for mutations in these apoptosis regulators. The importance of p53 in preventing cancer is highlighted by clinical data showing that it is functionally inactive in 50% of cancers, making it the most often mutated tumor suppressor (Fridman and Lowe, 2003). Additionally, both the anti- and pro-apoptotic Bcl-2 family members can be dysregulated in cancer to inhibit apoptosis (Adams and Cory, 2007).

1.2 TUMOR PROGRESSION

Ninety percent of cancer deaths are due to distant organ metastasis, making it the key event in tumor progression. The multistep process of metastasis is described as an invasion-metastasis cascade, consisting of initial local invasion, angio-/lymphangiogenesis, intravasation into blood and lymphatic vessels, transit through the vascular systems, extravasation into distant sites, formation of small non-proliferating colonies called micrometastases, and finally growth of these colonies to form macrometastases (summarized in Figure 2). In recent years, the understanding has

increased substantially of the timing of metastasis, complexity of the dissemination routes, and the intricacy of the mechanisms behind the organ tropism and dictation of micrometastasis growth (Sleeman et al., 2011).

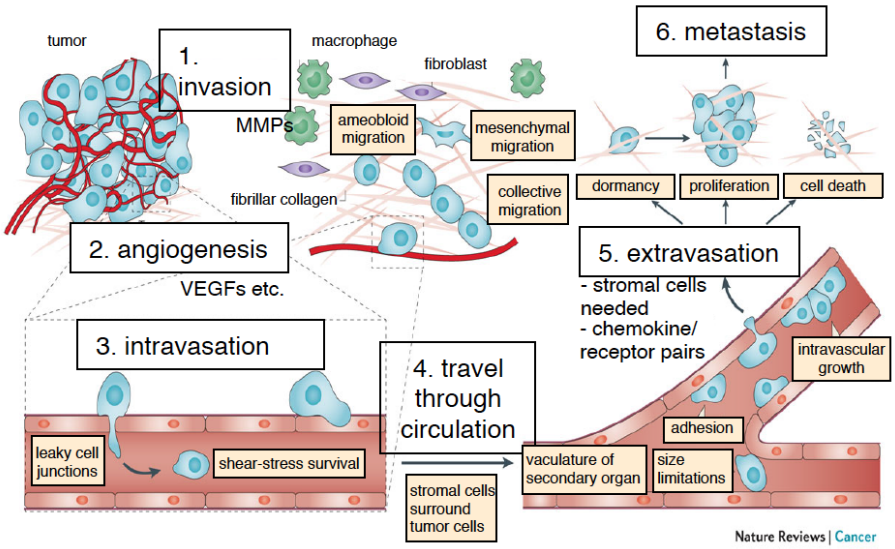

Figure 2. Invasion-metastasis cascade.

(modified from (Reymond et al., 2013))

1.2.1 Local invasion

When the tumor progresses from the pre-malignant stages to an invasive tumor, it needs the capability to invade into its surroundings. The key events in local invasion are changes in cell adhesion, activation of proteolysis, and increased motility, which can be achieved by inducing morphogenetic processes such as epithelial to mesenchymal transition (EMT, discussed more in chapter 2.1.2) (Hanahan and Weinberg, 2011). Tumor cells can migrate in two main ways, individually or collectively, and single-cell invasion can be further divided into amoeboid and mesenchymal invasion modes (Friedl and Alexander, 2011). Even though the invasion mode has been thought to be tumor type specific, it has lately been recognized that tumors can alternate between different migration patterns depending on their surroundings, i.e., the ECM composition and structural constraints or trails such as blood vessels (Haeger et al., 2014, Wolf et al., 2013). Active cell migration can be divided into five steps taking place in the individually migrating cell, or in the leading cell of the collectively invading mass (Friedl and Wolf, 2009). These steps include actin polymerization, adhering to the ECM via integrins, proteolysis mediated by metalloproteases such as matrix metalloproteinases (MMPs), contraction of the cellular cytoskeleton, and finally cell movement (Friedl and Wolf, 2009).

1.2.1.1 Matrix metalloproteinases (MMPs)

Matrix degrading proteases such as MMPs are key effectors of tissue invasion by cleaving the ECM components including collagens, non-collagenous glycoproteins (e.g. laminins, fibronectin), and proteoglycans (e.g. perlecan, decorin, or CD44) (Sevenich and Joyce, 2014). As the proteolytic cleavages by MMPs are irreversible events, it is not surprising that their gene expression, activity, and subcellular localization are strictly controlled (Kessenbrock et al., 2010). MMPs are activated from an inactive zymogen to an active enzyme by proteolytic removal of the pro-domain. Usually this is achieved by the activity of other proteases, such as plasmin, furin, or active MMP (Sternlicht and Werb, 2001). MMPs are either membrane bound (MT1-MMP-MT3-MMP) or secreted into the extracellular space (e.g. MMP1-MMP9) (Nagase et al., 2006). The active membrane bound enzymes localize to plasma membrane structures called invadopodia at high local concentrations (Murphy and Courtneidge, 2011), while secreted MMPs can act on the cell surface as well by binding to cell surface integrins and CD44 (Redondo-Munoz et al., 2008). MMP activity is additionally controlled by endogenous inhibitors such as tissue inhibitors of metalloproteinases (TIMPs) (Deryugina and Quigley, 2006). The proteases, their substrates and inhibitors form highly interconnected proteolytic networks, making the regulation of MMP activity extremely complex (Fortelny et al., 2014).

The expression of proteases is often upregulated in cancer and correlates to poor patient prognosis (Sevenich and Joyce, 2014). MMPs are mostly derived from the stromal cells in tumors (Egeblad and Werb, 2002), and they modify tumor progression in multiple ways. These include accelerated tumor growth, increased tissue remodeling, inflammation, tissue invasion, and metastasis (Kessenbrock et al., 2010). ECM remodeling leads to the formation of spaces for the invading cells, as well as to the generation of active ECM epitopes that promote cell adhesion and migration (Friedl and Alexander, 2011). In addition, MMPs can regulate the bioavailability of growth factors to tumor cells and endothelial cells (ECs) by cleaving the ECM and releasing the bound growth factors, thus inducing tumor cell proliferation and angiogenesis (Sevenich and Joyce, 2014). MMPs can also regulate the availability of cell surface molecules, including growth factor receptors (Overall and Blobel, 2007). MMPs have been shown to degrade cell adhesion molecules and intercellular junction proteins and, thus, further facilitate invasion. For example, MMP3 and MMP7 can cleave E-cadherin, mediate EMT, and reinforce the invasive capacity of epithelial tumor cells (Noe et al., 2001). In addition, MMPs are involved in intra- and extravasation in multiple ways. MMP17 has been shown to promote pericyte detachment from blood vessels, leading to vascular leakiness and tumor cell invasion to the vessels (Chabottaux et al., 2009). MMP1, on the other hand, has been shown to cleave Proteinase-Activated Receptor 1 (PAR-1) on ECs, which leads to loosened cell-cell contacts, thus increasing transendothelial migration of cancer cells (Juncker-Jensen et al., 2013).

1.2.2 Tumor angiogenesis

Vasculature is divided into two distinct but connected systems, the blood and the lymphatic vasculature. The function of the blood vasculature system is to provide oxygen and nutrients to cells, whereas the lymphatic vessel system functions as a gatekeeper of fluid homeostasis. In tumors, cancer cells use these systems additionally as highways for spreading, and therefore promote the formation of new vessels. Furthermore, increasing evidence shows that ECs actively interact with their surroundings and regulate tissue homeostasis for example by producing cytokines (discussed more in chapter 2.2.2).

1.2.2.1 Angiogenesis

Normally, the formation of new blood vessels is restricted to embryogenesis and some transient processes such as wound healing, and most of the vasculature in adults is in quiescent state. However, to enable tumor growth beyond 1 mm diameter, the tumors need to gain ability to grow new blood vessels from the pre-existing ones, i.e., activate sprouting angiogenesis in a process called 'angiogenic switch' (Hanahan and Folkman, 1996). The angiogenic switch occurs quite early in the tumorigenic process, as already non-invasive premalignant lesions show signs of it both in animal models and in human tissue (Raica et al., 2009). After initial activation of angiogenesis, different tumors show diverse patterns of neovasculature, nonetheless, the tumor vasculature is characteristically aberrant. The vessels exhibit extensive, serpentine sprouting and distorted, enlarged morphology. The blood flow is erratic, leading to insufficient supply of nutrients and removal of metabolites. In addition, the vessels are hyperpermeable, which leads to micro-hemorrhaging and accumulation of fibrin into the tumor, which assists tumor cell migration and subsequent stroma formation (Nagy et al., 2010).

The angiogenic switch is controlled by tilting the balance of pro-angiogenic factors and angiogenesis inhibitors secreted by the tumor cells or the tumor microenvironment towards induction of angiogenesis (Baeriswyl and Christofori, 2009). In tumors, the pro-angiogenic factors are generally expressed at high levels and inhibitors at low levels compared to corresponding normal tissues. The most profound pro-angiogenic factor is the vascular endothelial growth factor (VEGF-A), acting in collaboration with a plethora of other factors such as fibroblast growth factors (FGFs), platelet-derived growth factors (PDGFs), and Notch (Carmeliet and Jain, 2011). In tumor cells, there are several ways how VEGF-A expression is induced. These include cellular stress factors such as hypoxia inducible factor α (HIF-1α), as well as oncogenes and inactivated tumor suppressors (Baeriswyl and Christofori, 2009). VEGF-A signals through cell surface tyrosine kinase receptors (VEGFR-1-3) on endothelial cells. Mostly it mediates its angiogenic functions through VEGFR-2 (Koch et al., 2011). Activation of VEGFR-2 is initiated by dimerization to homo- or heterodimers with other VEGFRs, and auto- or transphosphorylation of specific tyrosine residues (Koch et al., 2011). This leads to phosphorylation and activation of downstream effectors along the PKC/ERK pathway, and finally to proliferation of the endothelial cells. Other biological responses to VEGFR-2 activation include migration, survival, and vascular permeability (Koch et al., 2011).

1.2.2.2 Lymphangiogenesis

In addition to the blood vascular system, the lymphatic system contributes to normal tissue homeostasis and cancer pathogenesis. In normal tissues, it has important functions in controlling interstitial fluid homeostasis, lipid absorption, and immune responses (Zheng et al., 2014a). Lymphatic capillaries have several characteristics which distinguish them from blood capillaries: they lack pericytes, have discontinuous basement membranes and button-like (cell-cell) intercellular junctions made of vascular endothelial cadherin (VE-cadherin) and other junction proteins (Alitalo, 2011). The capillaries drain into precollector vessels, sparsely covered by smooth muscle cells. The lymph then moves to the collecting lymphatic vessels, which are surrounded by smooth muscle cells and have basement membrane and continuous interendothelial cell junctions (Alitalo, 2011). Specific markers such as Prox-1, VEGFR-3, podoplanin and LYVE-1 distinguish the lymphatic ECs (LECs) from their vascular counterparts, blood ECs (BECs) (Albrecht and Christofori, 2011). Many of the markers have important functions in the embryonic differentiation of LECs from veins, as well as maintaining LEC identity. In embryonic veins, transcription factors SOX18 and COUP-TFII co-operate to activate Prox-1. Transcription factor Prox-1 is considered to be the master regulator of LEC fate (Johnson et al., 2008), and it controls the expression of other genes associated with LEC characteristics (Petrova et al., 2002). These genes include VEGFR-3, which is needed in sprouting angiogenesis towards its ligand VEGF-C. Final separation of veins and the lymphatic system in the embryo takes place with the aid of podoplanin, which triggers platelet aggregation and thus blocks the blood flow (Zheng et al., 2014a).

In adults, the main route to create new lymphatic vessels is sprouting from the pre-existing ones, in a process called lymphangiogenesis. Several factors influence this process, but VEGF-C/VEGFR-3 is the most important pathway. Binding of VEGF-C to VEGFR-3 leads to its dimerization and autophosphorylation. This leads to activation of downstream serine kinases AKT and ERK, and finally proliferation, migration and survival of LECs (Makinen et al., 2001). In addition, the VEGF-C stimulated angiogenesis is aided by many other secreted factors such as angiopoietin 2 (ANG-2), FGFs, and VEGF-VEGFR-2 axis. Lymphangiogenesis is additionally influenced by cell-cell contact mediated signaling via the Notch and ephrin pathways (Zheng et al., 2014a). Notch inhibition has been shown to synergize with VEGF to induce lymphangiogenesis in adult mice and in 3D cell models (Zheng et al., 2011), whereas EphrinB2 facilitates VEGF3 signaling (Wang et al., 2010). At the same time, lymphangiogenesis is balanced by endogenous inhibitors, including transforming growth factor β (TGF-β) and bone morphogenetic protein 2 (BMP-2) (Zheng et al., 2014b).

Similar to tumor blood vasculature, the tumor associated lymphatic vessels differ from their normal counterparts. High interstitial pressure inside the tumor leads to collapsed, poorly functional vessels, whereas in the periphery of the tumor the vessels are functional and thought to contribute to metastasis. In transcriptional profiling studies the tumor lymphatic vessels resemble activated, growing lymphatic vessels, and express markers of active lymphangiogenesis (Albrecht and Christofori, 2011). Additionally, LEC-LEC and

LEC-matrix interactions are altered in the tumors (Clasper 2008, Milteva 2010). The increased numbers of lymphatic vessels in the tumors and sentinel lymph nodes are mainly achieved by sprouting angiogenesis (Albrecht and Christofori, 2011). Lymphangiogenesis is initiated by tumor cells or stromal cells, especially tumor associated macrophages (TAMs), which express lymphangiogenic factors like VEGF-C and ANG-2 (Ji, 2012). In addition, other growth factors, including VEGF-A, FGF, PDGF-B, HGF, IGF-1, are overexpressed in the tumors and can induce lymphangiogenesis by more indirect mechanisms, for example by recruiting inflammatory cells (Zheng et al., 2014a). Incorporation of bone marrow derived progenitor cells have been described as another mechanism participating in the formation of new lymphatic vessels, albeit at low numbers (Patenaude et al., 2010).

1.2.3 Hematogenic spread and colonization to distant organs

In order to metastasize, cancer cells need to reach distant sites through one of the vascular systems. Spread via blood vasculature requires intravasation into the vessels, survival inside the vessels, and finally extravasation. As large numbers of cancer cells are shed into the circulation every day, but not all patients develop metastasis, it seems that intravasation is necessary but not sufficient for metastasis (Sleeman et al., 2011). In extravasation, cancer cells actively transmigrate through the capillary walls, while the endothelial junction proteins are downregulated and the ECs retract (Garcia-Roman and Zentella-Dehesa, 2013). For successful extravasation, a contribution from platelets, leukocytes, and macrophages is required (Gay and Felding-Habermann, 2011).

Many of the extravasated cells initially enter dormancy, but they can be activated to grow even decades after removal of the primary tumor. Analysis of tumor growth rates and single cell genomics of dormant tumor cells indeed suggest that tumor cell spread to distant organs is an early event (Husemann et al., 2008, Klein and Holzel, 2006), and the disseminated cells probably remain dormant before they gain ability to grow metastases (Klein, 2009). The signals that lead to the activation of dormant tumor cells are not fully understood, but modifications of the microenvironment to form a metastatic niche seem to play an important role. The central elements of the metastatic niche include perivascular location, modifications of the ECM, recruitment of bone marrow derived cells, hypoxia, and the expression of certain signaling molecules (Sleeman, 2012).

1.2.4 Contribution of the lymphatic system to metastasis

Lymphatic vessels can act as conduits for the tumor cells to metastasize. The high interstitial pressure in tumors and the high fluid flow facilitate invasion of tumor cells into the lymphatic vessels. The intrinsic properties of the lymphatic vessels, discontinuous contacts and smooth muscle coverage, and lack of basement membrane, make them fairly easily accessible to tumor cells. In addition, there is lower mechanical stress inside the lymphatic vessels than inside blood vessels. In human tumors, it has been observed that the lymphatic vessels can also host tumor cells themselves. *In transit* metastasis between the primary tumor and the sentinel lymph node is a likely example of this. Twenty percent

of melanoma patients who have undergone full primary tumor resection show signs of such lymphatic contribution (Alitalo, 2011). The status of lymph node metastasis correlates with prognosis, even though the lymph node metastases themselves usually are not fatal (Morton et al., 2006). In breast cancer and melanoma, which arise in tissues with abundant lymphatic vasculature, this is particularly clear. In these tumor types, draining or "sentinel" lymph node biopsy is used as part of clinical practice to facilitate prognosis and to establish treatment regime. However, the probability of distant organ metastasis and overall survival is not affected by the removal of sentinel or other draining lymph nodes (Sleeman et al., 2011). This suggests that lymph node metastasis does not serve as an additional source of disseminating tumor cells. To explain this paradigm, Klein and Holzel have suggested a parallel dissemination model, in which the tumors produce factors that can simultaneously activate lymphangiogenesis and act systemically to promote distant metastasis (Sleeman et al., 2009, Sleeman and Thiele, 2009, Klein and Holzel, 2006).

Mechanistically, tumor cells have been shown to interact with the lymphatic system in multiple ways: tumor cells can activate lymphangiogenesis, co-opt pre-existing lymphatic vessels, invade into them, and use factors secreted by the LECs as chemotactic clues (Sleeman and Thiele, 2009, Alitalo and Detmar, 2012). In some human cancer types, there are reports of a correlation between the density of lymphatic vessels, extent of lymphatic vessel invasion, and lymphangiogenic growth factor levels to lymph node and distant metastasis, and poor prognosis (Dadras et al., 2005, Dadras et al., 2003). This suggests that lymphangiogenic factors play an important role in metastasis. Indeed, data from animal models have shown that inhibition of VEGF-C/D-VEGFR3, COX-2, and PDGF-B activity reduces tumor-induced lymphangiogenesis, while the forced expression of VEGF-C/D induces lymph node and distant organ metastasis (He et al., 2002, Karpanen et al., 2001, Mandriota et al., 2001, Skobe et al., 2001, Stacker et al., 2001, Yanai et al., 2001). Tumor entry and metastasis can also be facilitated by the proliferation of LECs in the collective lymphatic vessels and lymph nodes by VEGF-A/C mediated mechanisms (Hirakawa et al., 2007, Hirakawa et al., 2005). Nevertheless, lymph node metastasis is not always associated with increased lymphangiogenesis. Especially in tissues with high lymphatic vessel content, tumor cells can co-opt pre-existing lymphatic vessels and metastasize without additional lymphangiogenesis (Sleeman et al., 2009). To invade into lymphatic vessels, tumor cells can secrete factors such as lipoxygenase, which downregulate LEC cell-cell contact molecules (Kerjaschki et al., 2011). Tumor lymphatic cells express specific adhesion molecules, including macrophage mannose receptor 1 (MR1) and CLEVER-1, which can be utilized by the tumor cells to attach to the lymphatic vessels and facilitate invasion (Irjala et al., 2003, Karikoski et al., 2014). Tumor cells can also hijack the immune cell attraction, adhesion, and homing mechanisms to the lymph nodes. This has been shown to occur through the chemokines such as CCL21 and CXCL12, secreted by the LECs, which attract tumor cells toward lymphatic vessels and support tumor growth (Aebischer et al., 2014, Hirakawa et al., 2009, Karaman and Detmar, 2014). Tumors can induce immunosuppression in the tumor draining lymph nodes by reducing the number and weakening the function of immune cells recognizing the tumor, thus further promoting tumorigenesis and impairing patient survival (Madsen, Sahai 2010).

2. CELLULAR REPROGRAMMING AND TUMOR-STROMA INTERACTIONS

It has become increasingly evident that tumors consist not only of a mass of mutated cancer cells but also of a vivid stromal component which interacts with the tumor cells and actively participates in the malignant process. Tumor stroma consists of basically the same elements as any other tissue, namely ECM, supporting cells such as fibroblasts, blood and lymphatic vessels, pericytes, and immune system components. However, the origin of the cells might differ from their normal counterparts, and their function is altered due to the active interaction with tumor cells (Quail and Joyce, 2013). The ECM can also promote tumorigenesis as it functions as a reservoir of cytokines and growth factors, the bioavailability of which is regulated by matrix degrading enzymes (Sevenich and Joyce, 2014). Moreover, tumor cells can mimic the properties of stromal cells and use these gained traits for their advantage in the malignant progression. Examples of such processes include transdifferentiation, in which a cell fate is converted from one differentiated cell type to another mature cell type (Campos-Sanchez and Cobaleda, 2014). The most well-characterized example of this is epithelial to mesenchymal transition (EMT), in which epithelial tumor cells adapt characteristics of mesenchymal cells to help their invasion into the surrounding tissues (Thiery, 2002). Tumor cells can additionally mimic other stromal components, for example, ECs in a process called vascular mimicry. This is a source of functional blood vessels in the tumor, leading to better survival of the tumor cells (Seftor et al., 2012). Furthermore, tumor cells have been described to be able to dedifferentiate, i.e., revert the normal differentiation and give rise to progenitors of the same phylogenic tree. These cells serve as reservoirs to produce more differentiated cancer cells, and are sometimes called cancer stem cells (Campos-Sanchez and Cobaleda, 2014). The tumor cells are not solely capable of differentiating into the stromal cells, but the stromal cells can also transdifferentiate into other stromal cell types. ECs can serve as sources for cancer associated fibroblasts through a process called endothelial to mesenchymal transition (EndMT) (Potenta et al., 2008), and macrophages have been shown to be able to transdifferentiate into LEC progenitors, and possibly contribute to the tumor lymphangiogenesis (Patenaude et al., 2010).

2.1 CELLULAR REPROGRAMMING IN CANCER

2.1.1 Oncogenes induce cell fate changes

The terminal differentiation of cells restricts cell proliferation by instructing the cells to enter irreversibly into a post-mitotic state. In cancer, oncogenes can hinder the differentiation programs, i.e., reprogram the cell, to favor cell proliferation. The best-characterized example is the c-Myc oncogene, which is overexpressed in many cancers leading to a proliferative phenotype. Conditional mouse models have shown that if c-Myc expression is shut down in tumors, the cancer cells can enter a terminal differentiation program, senescence, or apoptosis (Gabay et al., 2014). This suggests that in some cancers constitutive oncogene expression is needed to prevent the cells from differentiating and going into a non-proliferative state. Furthermore, cancer can be seen as a set of oncogenic alterations leading to reprogramming of the normal cellular identity to form a new

pathogenic lineage (Campos-Sanchez and Cobaleda, 2014, Goding et al., 2014). Studies on the reprogramming of cells to pluripotency have highlighted the similarities between cancer progression and generation of induced pluripotent stem cells (iPSC). In both, the cells acquire unlimited proliferation and self-renewing abilities (Semi et al., 2013). Additionally, the original four reprogramming transcription factors, c-Myc, Oct4, Klf4, and Sox2, needed to reprogram fibroblasts into iPSCs (Takahashi and Yamanaka, 2006), were already earlier described to have oncogenic capacities. The establishment of these factors in the induction of pluripotency further emphasized that despite the complex interplay between transcription factors and the epigenetic landscape in maintaining the cellular identity, a limited number of cellular genes can fully bypass these mechanisms. This suggests that in cancer as well the function of only a few oncogenes can be sufficient to change the fate of the cell, but in a cell type dependent manner. Other studies have indeed shown that an alteration in the expression of a single transcription factor might even be enough for cell fate changes such as dedifferentiation and transdifferentiation. For example, elimination of Pax5 expression, which is the driver of B-cell identity, leads to the dedifferentiation of B-cells to hematopoietic progenitors and aggressive progenitor cell lymphomas (Cobaleda et al., 2007).

2.1.2 Mesenchymal transitions

2.1.2.1 Epithelial to mesenchymal transition (EMT)

EMT is characterized as replacement of the quiescent epithelial phenotype by an invasive and migratory mesenchymal phenotype (summarized in Figure 3). In normal development, repetitive rounds of EMT and the opposite process, mesenchymal to epithelial transition (MET), are necessary for completing the gastrulation and primitive streak formation. In addition, EMT is needed for the cells to migrate to different sites in the body during embryogenesis (Nieto, 2013). Tumor cells have adapted to use these morphogenic processes to spread. EMT can mostly be seen at the borders of the tumor, and it gives rise to invasive and migratory cells that intrude into the surrounding stroma. The reversibility of the process is also used by the tumor cells: EMT allows them to leave the primary tumor site, whereas MET is used by them to colonize at the metastatic sites (Thiery, 2002). EMT is defined by the loss of expression of the cell-cell contact markers, such as E-cadherin and tight junction proteins, and the gain of expression of mesenchymal markers, including vimentin, fibronectin, fibroblast specific protein (FSP-1), alpha smooth muscle actin (α-SMA), and N-cadherin (Kalluri and Weinberg, 2009). As the cell cytoskeleton is rearranged as well, the cell shape changes to spindle like, resembling fibroblasts. The initiation of these processes is controlled by specific growth factors and cytokines, hypoxia through HIF-1α, as well as contacts with the ECM (Gonzalez and Medici, 2014). These signals work in cell and tissue type specific manner, and lead to activation of signaling pathways such as TGF-β, BMP, FGF, PDGF, and Notch (Espinoza and Miele, 2013, Heldin et al., 2012, Katoh and Katoh, 2009, McCormack and O'Dea, 2013). The initiated intracellular kinase cascade leads to activation of specific transcription factors, which mediate the EMT process. The transcription factors include Snail, Slug, Twist, and ZEB1/2, which have been shown to act on the E-cadherin gene

(*CDH1*) promoter and inactivate it (Peinado et al., 2004, Yang et al., 2004). In addition, the same factors can repress the epithelial adherent junction proteins, leading to dissociation of the cell-cell contacts (Eger et al., 2005, Vandewalle et al., 2005). ZEB1/2 has also been shown to increase the expression of MMPs and subsequently the invasive and migratory capacity of the cells (Miyoshi et al., 2004).

2.1.2.2 Endothelial to mesenchymal transition (EndMT)

ECs exhibit diversity in their gene expression depending not only on their localization in the vascular tree but also on the tissue environment (Chi et al., 2003). An example of EC plasticity beyond the endothelial fate is EndMT, where the endothelial cell features are replaced by the mesenchymal phenotype (see Figure 3 and (Armstrong and Bischoff, 2004)). EndMT is a reprogramming program sharing characteristics with EMT (Saito, 2013). Similar to EMT, EndMT occurs in the normal development. EndMT is best studied in the developing heart, where it takes place in the formation of the valves and septa from the endoderm (Garside et al., 2013). EndMT can additionally function in pathological conditions, including cancer and cardiac fibrosis (Zeisberg et al., 2007a, Zeisberg et al., 2007b, Potenta et al., 2008), as well as contribute to the formation of the mesenchymal stem cell phenotype (Medici and Kalluri, 2012, Medici et al., 2010), which can further give rise to pathological ossification (Medici and Olsen, 2012). In cancer, *in vivo* studies have suggested that EndMT can serve as a significant source of cancer associated fibroblasts (CAFs), which have an established role in tumor progression (Zeisberg et al., 2007a). The characteristics of EndMT include losing the EC markers (PECAM, Tie1, Tie2, VEGFR), loosening of the endothelial junctions (VE-cadherin), gaining markers of the mesenchymal cells (FSP-1, α-SMA, fibronectin, vitronectin, collagen types I and II), and increasing the invasive and migratory properties (Potenta et al., 2008). Most of the studies on EndMT regulation have concentrated on developmental EndMT, and shown that it can be induced by the coordinated function of TGF-β, Notch, and BMP pathways (Garside et al., 2013). For example, the conditional mouse knockouts of TGF-β2, BMP-2 and BMP-4 are defective for EndMT induced phenotypes (Azhar et al., 2009, Ma et al., 2005, McCulley et al., 2008). Additional pathways, including VEGF, NFAT, Wnt, ErbB and NF1/Ras, have also been implicated to be involved in EndMT associated with heart development (Armstrong and Bischoff, 2004). Downstream effects, such as the downregulation of VE-cadherin expression, seem to be mediated by the Snail family of transcriptional repressors (Medici et al., 2011). However, their expression is not sufficient to cause EndMT: It has been recently shown that Slug expression in ECs can lead only to partial EndMT. This further leads to MT1-MMP expression and angiogenic sprouting (Welch-Reardon et al., 2014a, Welch-Reardon et al., 2014b).

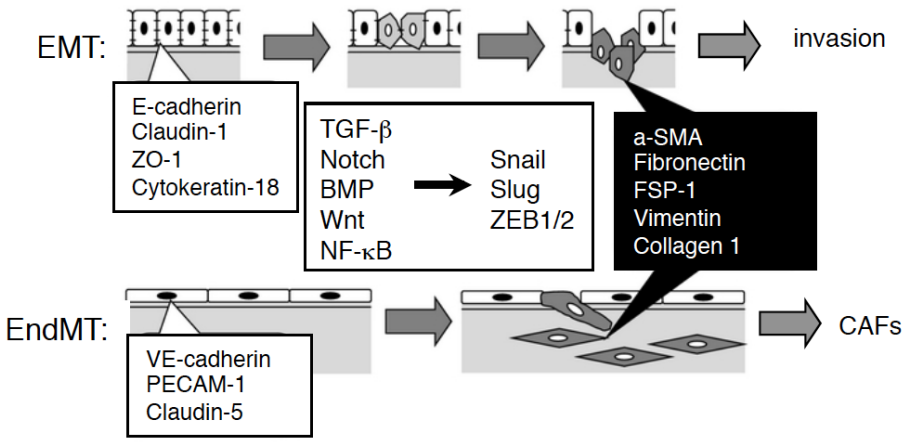

Figure 3. EMT/EndMT.

Morphogenic processes EMT and EndMT result in loss of epithelial/endothelial markers (indicated in the two white boxes on the left) and gain of mesenchymal markers (listed in the black box on the right). Finally, this leads to increased cancer cell invasion and can give rise to CAFs. TGF-β, Notch, BMP, Wnt, and NF-κB signaling have been shown to induce EMT/EndMT through transcription factors Snail, Slug, and ZEB1/2 (modified from (Miyazono, 2009)).

2.1.3 Selected cellular pathways deregulating differentiation in cancer

2.1.3.1 Notch signaling pathway

The Notch pathway has been implicated to be important in regulating proliferation, differentiation and survival/apoptosis both in development and pathological conditions such as cancer (Guruharsha et al., 2012). Furthermore, it has been shown to be involved in the maintenance of the stem cell properties, angiogenesis, and morphogenic processes such as EMT and EndMT (Dzierzak and Speck, 2008, Kofler et al., 2011, Espinoza and Miele, 2013). In mammals, there are four Notch receptors (Notch1-4), which bind ligands belonging to the delta like (Dll1 and Dll3-4) or Jagged (Jag1-2) families. As both the receptors and ligands are membrane bound, the pathway activation needs cell-cell contact between ligand expressing and receptor expressing cells. The ligand binding results in a conformational change of the receptor, allowing disintegrin and metalloproteinase 10 and/or 17 (ADAM10 and/or ADAM17) to cleave the extracellular part of the receptor. This subsequently enables γ-secretase enzyme to cleave the receptor from the intracellular side resulting in formation of Notch intracellular domain (NICD), which can then translocate to the nucleus. There it binds transcription factor RBP-Jκ, which allows RBP-Jκ dissociation from its repressor complex, and association with transcription activators such as MAML and p300 (reviewed in (Lobry et al., 2014)). The formed complex acts as transcriptional regulator of the Notch pathway targets, including the most well characterized hairy enhancer of split (Hes) family of transcription repressors, the Notch-related ankyrin repeat protein (Nrarp), c-Myc, and cyclin D1 and D3 (Borggrefe and

Oswald, 2009). In addition, the Notch pathway has been shown to regulate a large number of additional genes, many of them cell-type specific (Borggrefe and Oswald, 2009, Hamidi et al., 2011). The Notch pathway activation is summarized in Figure 4, which additionally shows the KSHV regulation of Notch described in more detail in chapter 3.1 and ref. (Cheng et al., 2012).

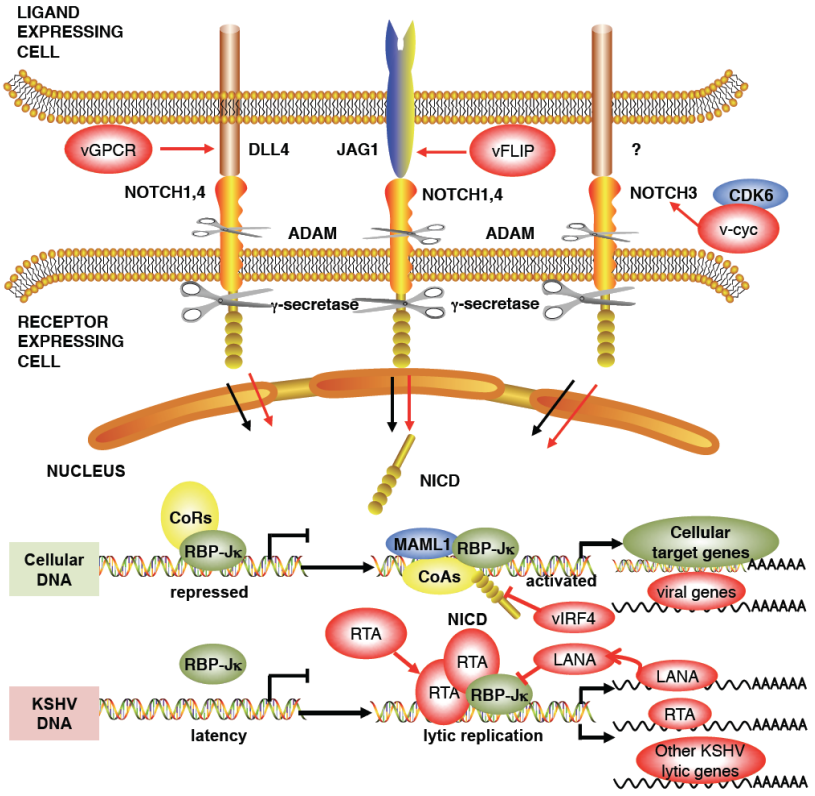

Figure 4. Notch pathway activation, and how it is steered by KSHV.

Summary of Notch pathway activation is show in bold, while the KSHV regulators of the Notch pathway are with red background. CoR= transcription co-repressors; CoA= transcription co-activators; modified from (Cheng et al., 2012).

2.1.3.1.1 Notch signaling in differentiation and tumorigenesis

The role of Notch pathway has been studied most profoundly in the hematopoietic system, where Notch signaling has been shown to be an essential regulator of the proliferation, self-renewal, and differentiation of the hematopoietic stem and progenitor cells. Notch pathway activation is sufficient for the cell fate determination of T-cells over B-cells, as DLL4 activates T-cell differentiation processes of the primary human CD34+ cells (Lefort et al., 2006). In addition, Notch-RBP-Jκ regulates the differentiation of marginal zone B-

cells as well as αβ–T-cells (Tanigaki et al., 2002, Tanigaki et al., 2004). Moreover, the imbalance in the Notch signaling can lead to alteration in these processes causing transformation (Kushwah et al., 2014).

Regulation of cell differentiation by Notch is compatible with the diverse role of Notch in different malignancies. It has been found to be oncogenic in many of the cell types where its activity is needed for differentiation. The most profound example of this is the role of activated Notch in T-cell leukemo-/lymphomagenesis (Aifantis et al., 2008). In humans, activating mutations in *NOTCH1* have been described in 56% of human T-cell acute lymphoblastic leukemia (T-ALL), making *NOTCH1* the most frequently mutated gene in T-ALL (Weng et al., 2004). Most probably, these mutations lead to *MYC* oncogene activation and inactivation of the tumor suppressors p16 and p14 in hematopoietic progenitors, which leads to differentiation towards T-cell development (Ferrando et al., 2002). In mouse models, the oncogenic *NOTCH1* mutations have been shown to accelerate K-RAS induced transformation of normal T-cells (Chiang et al., 2008). Additionally, overexpression of NICD1 and NICD3 has been shown to lead to T-cell lymphoma formation (Aster et al., 2000, Izon et al., 2001, Pear et al., 1996). Notch has also been shown to be oncogenic in other contexts, such as breast cancer and melanoma (Koch and Radtke, 2007, Pinnix and Herlyn, 2007). Notch activates pathways involved in the initiation of these tumor types, including AKT and NF-κB (Liu et al., 2006, Bedogni et al., 2008, Osipo et al., 2008), and has been shown to have a role in maintaining cancer stem cells (Fan et al., 2010, Wang et al., 2011). However, Notch activation has also been linked to tumor suppressive functions in some cancers, especially in skin squamous cell carcinoma where Notch1 activates tumor suppressor p21 (Rangarajan et al., 2001). Taken together, Notch has a pivotal role in tumor formation depending on the type of the malignancy, genetic landscape, and mechanisms that are still inadequately understood.

2.1.3.1.2 Notch in angiogenesis and mesenchymal transitions

In addition to the direct effects on the tumor cells and stem cells, Notch signaling plays an additional role in regulating normal and tumor angiogenesis (Benedito and Hellstrom, 2013). ECs express Notch1, Notch2, and Notch4, as well as ligands Dll1, Dll4, and Jag1 (Kofler et al., 2011). The importance of the activated Notch in developmental angiogenesis is elucidated by genetic knockout experiments, in which loss of *Notch1, Hey1/2, or Rbp-jκ* has led to embryonic lethality due to defects in sprouting angiogenesis (Fischer et al., 2004, Krebs et al., 2004, Krebs et al., 2000). Dll4 and Notch levels are interchangeably regulated by the VEGF-VEGFR2 axis. This arrangement is needed to give rise to appropriate numbers of VEGF responsive cells, organized angiogenesis, and finally functional vessels (Jakobsson et al., 2010). In the tumor vasculature, the inhibition of Dll4/Notch1 axis has been accordingly shown to suppress tumor growth by giving rise to hyper dense immature vascular network of non-functional vessels (Noguera-Troise et al., 2006, Ridgway et al., 2006).

Besides the ECs, Notch regulates the vessel mural cells, which play a supportive role in normal and pathological angiogenesis (Benjamin et al., 1998). Notch3 has been shown to

be necessary for the maintenance of the mural cells (Domenga et al., 2004, Liu et al., 2010), and to be induced by Jag1 expressed by the ECs (Liu et al., 2009). In addition, activated Notch can upregulate PDGFR-β expression leading to enhanced mural cell recruitment (Jin et al., 2008). Dll4 is then needed in the bone-marrow recruited cells to express pericyte/vascular smooth muscle cell markers (Stewart et al., 2011). Furthermore, Notch activation has been shown to promote EndMT and EMT by directly upregulating PDGFR-β and α-SMA (Jin et al., 2008, Noseda et al., 2006), the EMT/EndMT transcription factors Snail and Slug (Niessen et al., 2008, Sahlgren et al., 2008), and repressing E-cadherin gene expression (Becker et al., 2007, Leong et al., 2007). Additionally, Notch dependent transcription factors Hif-1α, Msx1, Sox9, and Stat4 were shown to drive EndMT in cardiac valve development (Chang et al., 2014). Notch also interacts with other pathways, such as TGF-β, in inducing EMT/EndMT. TGF-β has been shown to induce Notch ligands (Niimi et al., 2007), and TGF-β induced EMT can be blocked by Notch inhibition (Zavadil et al., 2004).

2.1.3.2 Nuclear factor kappa B (NF-κB) pathway

Nuclear factor kappa B, NF-κB, transcription factor family regulates inflammation and cancer in a complex manner and consists of five members including p65 (RelA) (Hoesel and Schmid, 2013). These factors form homo- or heterodimers, and in quiescent cells they are bound to inhibitors of the IκB family (May and Ghosh, 1997). The NF-κB gene expression activation requires upstream kinase signaling that leads to IκB phosphorylation and ubiquitinylation, release of the transcription factors from the inhibitors, and shift of the transcription factor dimers to the nucleus (Hayden and Ghosh, 2008). The kinase cascade is initiated by the ligand binding of membrane bound receptors, including Toll-like receptors (TLRs) stimulated by microbial components, interleukin 1 (IL-1) receptor, and TNF receptor (Schmid and Birbach, 2008). When the NF-κB transcription factors bind DNA, it leads to transcription of the target genes, including anti-apoptotic genes such as Bcl-2, cytokines including IL-1 and IL-6, as well as adhesion molecules such as VCAM-1 and ICAM-1 (Hoesel and Schmid, 2013). Different dimers have different DNA binding preferences, which can be altered by post-translational modifications such as phosphorylation (Oeckinghaus and Ghosh, 2009). For example, serine 536 (S536) phosphorylation of p65 has been shown to stimulate its transcriptional activity and stability, as well as decrease its nuclear export (Bohuslav et al., 2004, Delhase et al., 2012, Lawrence et al., 2005). These phosphorylations serve as points of crosstalk with other signaling pathways, such as the mitogen-activated protein kinase (MAPK) pathway (Schulze-Osthoff et al., 1997). Furthermore, NF-κB members both positively and negatively interact with other transcription factors, such as STAT3 and p53, as well (Grivennikov and Karin, 2010, Webster and Perkins, 1999).

Diverse mechanisms can lead to constitutively active NF-κB in tumors. These include direct mutations, oncogene mediated activation, and tumor microenvironment secreting cytokines. In some hematopoietic malignancies, it has been seen that oncogenic p65 mutations can drive the tumorigenesis (Courtois and Gilmore, 2006). In contrast, in many solid tumors, NF-κB activity is maintained by constitutive flux of cytokines from tumor-

associated macrophages (Hagemann et al., 2008). The role of NF-κB in cancer is multidimensional, as it can be both anti- and pro-tumorigenic (Hoesel and Schmid, 2013). By mediating acute inflammatory response, its activation can lead to cytotoxicity towards tumor cells (Disis, 2010). However, experiments on genetically modified mice suggest that active NF-κB has a role in the survival of various tumors. For example, N-Ras induced mouse melanoma initiation required IKK2-mediated NF-κB activation (Yang et al., 2010). The constitutive activation of NF-κB seen in many tumors can act in a protumorigenic manner via multiple mechanisms. In addition to innate immunity, NF-κB activation is linked to increased survival by regulating cell proliferation and apoptosis (Guttridge et al., 1999, La Rosa et al., 1994). NF-κB has also been shown to control EMT and upregulate VEGF, leading to increased angiogenesis, invasion and metastasis (Huber et al., 2004, Xie et al., 2010), as well as to stimulate lymphangiogenesis (Flister et al., 2010, Ji, 2014).

2.2 TUMOR-STROMA INTERACTIONS

2.2.1 The pivotal role of cancer associated fibroblasts (CAFs)

Cancer associated fibroblasts (CAF) are activated fibroblasts residing within the tumor. They share similar properties with fibroblasts involved in inflammation and wound healing (Madar et al., 2013). They express a variety of activated stromal cell markers such as FSP-1, fibroblast activating protein (FAP), vimentin, and α–SMA, as well as receptors for the PDGFs (Augsten, 2014). However, none of these markers is specific for the CAFs as the wide variety of normal fibroblasts and other stromal cells also express some of these markers, which makes distinguishing between these cells difficult (Madar et al., 2013). The diverse origin of CAFs contributes to their heterogeneity. CAFs can arise from tissue-resident fibroblasts stimulated by the tumor cells, recruited bone marrow cells (BMCs), or through EMT/EndMT (Elkabets et al., 2011, Quante et al., 2011, Radisky et al., 2007, Zeisberg et al., 2007a). α–SMA-expressing pericytes and adipocytes have also been suggested as possible origins of CAFs (Bochet et al., 2013, Dulauroy et al., 2012, Goritz et al., 2011).

The role of CAFs in promoting tumor progression is well established. They differ from normal fibroblasts as they are more proliferative and produce more collagen, and a myriad of cytokines, chemokines, and growth factors (Augsten, 2014). These affect in autocrine and paracrine manners, and promote tumor growth, invasion and EMT, as well as recruit other stromal cells such as ECs and immune cells (Orimo et al., 2005, Augsten et al., 2009). In addition, CAFs are able to remodel the ECM by expressing matrix components and matrix-remodelling enzymes including MMPs (Spaeth et al., 2009, Sugimoto et al., 2006). CAFs have shown tumor promoting potential in mice when they are recruited to the tumor site, when they are co-injected with tumor cells, or when the injected tumor cells are pre-conditioned by CAF media (Olumi et al., 1999, Suetsugu et al., 2011). CAFs promote tumor progression from premalignant phase, stimulate metastasis and support outgrowth of disseminated cells (Malanchi et al., 2012, Zhang et al., 2013). However, the function of CAFs is context dependent, as they have also been shown to have tumor

suppressing functions (Chang et al., 2012, Green et al., 2013). It is plausible that the tumor cells and the tumor microenvironment modulate the function of CAFs and determine the CAF function and marker expression (Augsten, 2014).

2.2.2 Endothelial cells as tumor regulators

Lately, it has been recognized that ECs do not just form conduits for liquids and cells but can participate in regulating tissue homeostasis in various settings. Hu et al. showed that liver regeneration is regulated spatiotemporally by liver ECs. They produce different levels of ANG-2 depending on the phase of liver regeneration and coordinate hepatocyte and EC proliferation in response to injury (Hu et al., 2014). Furthermore, ECs have been described to both positively and negatively regulate the tumor cell survival and growth. Tumor ECs are important in the maintenance of glioblastoma stem cells via Notch signaling in 3D culture models (Hovinga et al., 2010), whereas endothelially expressed DLL4 has been shown to inhibit small cell lung cancer cell growth by activating Notch1 in the tumor cells (Ding et al., 2012). Additionally, tumor cell – EC interactions have been shown to modify their reciprocal adhesion properties. Tumor cells expressing CD44 stimulate/upregulate E-selectin expression on the surface of ECs, which ultimately leads to expression of ICAM-1 on the surface of ECs. Upregulated ICAM-1 levels can in turn recruit neutrophils and assist melanoma cell adhesion to ECs, as well as enhance their migration (Zhang et al., 2014). Furthermore, ECs can express chemokines attracting tumor cells and thus enable metastasis to lymph nodes and distant organs. For example, chemotaxis towards lymphatics has been shown to be mediated by the interaction between LEC expressed CXCL12, CCL1, and CCL21 ligands and CXCR4, CCR8 and CCR7 chemokine receptors expressed by many tumor cells (Cabioglu et al., 2005, Shields et al., 2007, Das et al., 2013). The reciprocity and complexity of tumor cell – EC interactions are well demonstrated in studies by Lee and colleagues: they show that breast cancer secreted factor, IL-6, can condition lymphatic ECs at pre-metastatic sites by Stat3 phosphorylation in LECs. This leads to increased angiogenesis via HIF-1α and VEGF induction. Additionally, CCL5 expression is upregulated in the tumor conditioned LECs, leading to recruitment of tumor cells to metastatic sites and thus increased metastasis (Lee et al., 2014a, Lee et al., 2014b). Furthermore, it has been recently shown that ECs can promote metastasis by recruiting tumor promoting immune cells to the pre-metastatic niche by secreting factors such as ANG-2, and that this can be attenuated by anti-ANG-2 therapy (Srivastava et al., 2014).

2.2.3 Immune system in tumor progression

In addition to CAFs and ECs, components of the immune system are important regulators of tumor initiation and progression. Immune cells can be either inhibitory for tumor growth or they can promote the tumors. Anti-tumorigenic cells include CD8+ T-cells and natural killer cells. It has been shown that high CD8+ T-cell count in some tumor types correlates with better prognosis (Azimi et al., 2012, Galon et al., 2006). However, established tumors hardly ever encounter immune system mediated rejection, suggesting that the tumor microenvironment in general is immunosuppressive (Gajewski et al., 2013).

Indeed, several mechanisms for immune evasion have been described. These include dysfunction of CD8+ T-cells, accumulation of regulatory T-cells, switching to inhibitory dendritic cell subsets, and immunosuppressive function of other stromal cells (Gajewski et al., 2013). Furthermore, macrophages can act as tumor promoters in primary tumors as well as in metastases (Noy and Pollard, 2014). The tumor associated macrophages (TAMs) are characterized by production of immunosuppressive molecules such as TGF-β and are able to inhibit effector immune cells by a myriad of other mechanisms as well (Noy and Pollard, 2014). TAMs can additionally contribute to tumor promotion by producing growth factors for the tumor cells (Goswami et al., 2005), TGF-β secreted by them can induce EMT (Bonde et al., 2012), and they can promote angiogenesis and hematogenous metastasis by several mechanisms, including production of VEGF (Coffelt et al., 2009, Yeo et al., 2014). In the metastases, macrophages can aid tumor extravasation, promote tumor cell survival, induce angiogenesis, and inhibit cytotoxic T-cell function (Noy and Pollard, 2014).

3. NON-EPITHELIAL SKIN CANCERS

3.1 KAPOSI'S SARCOMA HERPESVIRUS (KSHV) AND ASSOCIATED MALIGNANCIES

Kaposi's sarcoma (KS) was first characterized by Moritz Kaposi in 1872 as an idiopathic pigmented sarcoma of the skin (Sternbach and Varon, 1995). It remained a rather rare and unknown entity until early 1980s, when its prevalence suddenly rose among the homosexuals of the US West Coast (Friedman-Kien, 1981). This led to a hypothesis of infectious origin of the disease, and KS was found to be linked to the human immunodeficiency virus (HIV) infection and acquired immunodeficiency syndrome (AIDS). However, it was not until 1994, when herpesvirus DNA was found from the KS lesions, that KSHV was discovered (Chang et al., 1994). Further studies showed that KSHV is necessary but not sufficient to cause the KSHV associated malignancies: KS (Chang et al., 1994, Moore and Chang, 1995), primary effusion lymphoma (PEL) (Cesarman et al., 1995a, Nador et al., 1995), and multicentric Castleman's disease (Soulier et al., 1995). The genome of KSHV consists of double stranded DNA (dsDNA), and it belongs to the *Gammaherpesvirinae* subfamily of the *Herpesviridae* family. KSHV consists of capsid that contains the big genome, encoding more than hundred open reading frames (ORFs), surrounded by amorphous protein tegument and a lipid envelope (Neipel et al., 1998). The vast genome contains remarkable number of genes hijacked from the ancient host cells, including homologs of cyclin D, Flice inhibitory protein (FLIP), and G-protein coupled receptor (Jarviluoma and Ojala, 2006), which has made it a valuable tool in cancer biology research. The consequences of the KSHV oncogene expression for regulating the virus replication, and the cancer hallmarks are summarized in Figure 5.

V-ONC	PATHWAY	CONSEQUENCE
vFLIP	NF-kB, Notch	
LANA	p53, Rb, HIF, Notch	
vGPCR	PI3K-Akt-mTOR, ERK, p38, JNK, NF-kB, Notch	
vIRF1	aIFN, p53, ATM, Bim	
v-cyclin	cell cycle, Notch	

	cancer hallmarks	virus replication
	resist cell death	maximize virus production, multiply latent episomes
	recruit or inhibit DDR	ensure correct replication
	inducing angiogenesis	find/create conditions for replication
	induce the cell cycle	find/create conditions for replication
	deregulating cellular energetics	find/create conditions for replication
	activate invasion and metastasis	
	evade growth suppressors	multiply latent episomes
	enabling replicative immortality	multiply latent episomes
	avoiding immune destruction	maximize virus production
	tumor promoting inflammation	multiply latent episomes

Figure 5. KSHV Replication and Persistence Strategies.

KSHV oncogenes, as well as cellular signaling pathways and cancer hallmarks that they regulate are shown in the upper panel. The cancer hallmarks are depicted by the color code in the lower table, which also shows the consequence for virus replication. DDR= DNA damage response (modified from (Mesri et al., 2014)).

3.1.1 Virus life cycle in KSHV pathogenesis

Like other herpesviruses, KSHV exhibits two phases of infection: latency and lytic replication. In latency, the virus hides from the host immune system and only a few viral genes are expressed, namely latency associated nuclear antigen (LANA), viral homolog of FLIP (vFLIP), viral cyclin D homolog (v-cyclin), viral interferon regulatory factor 3 (vIRF3), kaposin B, and the KSHV microRNAs (miRNAs) (Dittmer et al., 1998, Sin and Dittmer, 2013). In latently infected cells, the viral genome is found as a separate circular DNA, called episome. The episome is dividing together with the host genome during mitosis, as KSHV LANA keeps them attached together (Hu et al., 2002, Verma et al., 2006). For the virus to rapidly replicate, the virus needs to enter the lytic phase of the life cycle by a process called reactivation. This can be initiated by physiological stimuli such as hypoxia or by chemicals affecting the epigenetic regulators of the host (Ye et al., 2011, Yu et al., 1999). In lytic replication, the whole viral genome is transcribed in an organized manner finally leading to production of new viral particles and lysis of the host cell (Sun et al., 1999).

As KSHV is in latency in most tumor cells, and tumorigenesis is a time-consuming process, it has been thought that the latent genes are especially important in the

development of the cancer (Giffin and Damania, 2014). Supporting this, expressing the latency locus in mouse B-cells leads to plasma cell hyperplasia and lymphoma in a small subset of cases (Sin and Dittmer, 2013). Most of the latent KSHV genes have been shown to have oncogenic potential in *in vitro* cell models, and many of them also in transgenic mouse models. The cellular pathways affected are varied but involve pathways related to cell survival and growth (see Figure 5). For example v-cyclin is a viral D cyclin homolog (Chang et al., 1996), and LANA inactivates negative regulators of the cell cycle, p53 and pRb (Friborg et al., 1999, Radkov et al., 2000). vFLIP and miRNAs on the other hand have been shown to inhibit apoptosis (Liu et al., 2002, Suffert et al., 2011). vFLIP has also been shown to affect cell survival pathways, such as NF-κB (Guasparri et al., 2004, Keller et al., 2000). It is not surprising that latent viral genes affect these pathways, as it is crucial for the survival of the virus that the host cell stays alive as long as the virus is not replicating. Thus, the cancer appears to be rather a side effect of the viral life cycle. There is evidence of the oncogenicity of v-cyclin, vFLIP, and LANA in transgenic mouse models as well (Ballon et al., 2011, Fakhari et al., 2006, Verschuren et al., 2004a, Verschuren et al., 2002). However, some of the oncogenes, including K1, K15, viral G-protein coupled receptor (vGPCR), viral IL-6 (vIL6), and viral Bcl-2 homolog (vBcl-2), are expressed only during the lytic phase (Giffin and Damania, 2014). The lytic cycle is additionally needed to infect new target cells in tumors, and it has been shown that increased lytic replication and viral load correlate with poor prognosis. Moreover, high lytic replication level has been shown to cause resistance to therapies (El Amari et al., 2008, Nichols et al., 2011). Below, some of the most important viral oncogenes are introduced.

3.1.1.1 v-cyclin

The latent KSHV transcripts LANA, vFLIP and v-cyclin are transcribed from a common transcription start site. After splicing, LANA is expressed separately, but vFLIP and v-cyclin are expressed from a common bicistronic message (Dittmer et al., 1998, Talbot et al., 1999). KSHV viral cyclin (v-cyclin) is a homolog of cellular cyclin D. At sequence level, it mostly resembles cyclin D2, but shares homology with cyclin D1 and D3 as well (Chang et al., 1996). It has been shown to bind to many different cyclin dependent kinases (CDK) *in vitro*, but all its functions known to date are mediated by CDK6 (see Figure 6). Similar to cyclin D-CDK6 interaction, the binding of v-cyclin with CDK6 leads to activated kinase function of CDK6 and phosphorylation of its targets, most notably the retinoblastoma protein (pRb), and G1 to S-phase cell cycle progression (Godden-Kent et al., 1997, Verschuren et al., 2004b). Furthermore, v-cyclin has functions beyond cyclin D. Together with CDK6, it can phosphorylate multiple targets of S-phase cyclin E-CDK2 complex including histone H1, CDC6, ORC1 (Ellis et al., 1999, Laman et al., 2001, Mann et al., 1999), and Bcl-2 (Ojala et al., 2000). As the v-cyclin expression from the viral genome is constitutive, normal cell cycle regulation cannot control it. In addition, v-cyclin-CDK6 can deregulate cell cycle by inactivating the cell cycle inhibitors p21 and p27 by specific phosphorylations (Ellis et al., 1999, Mann et al., 1999, Sarek et al., 2006, Swanton et al., 1997). Taken together, active v-cyclin-CDK6 complex leads to progression of cell cycle in a manner that is not controlled by the cellular regulation mechanisms. Like

other oncogenes, extensive v-cyclin expression without compensatory (viral) mechanisms leads to oncogene induced DNA damage response and senescence (Koopal et al., 2007), autophagy (Leidal et al., 2012), as well as cell cycle arrest and apoptosis (Koopal et al., 2007, Ojala et al., 2000, Verschuren et al., 2002, Ojala et al., 1999). Further proof for the oncogenic function of v-cyclin comes from the rat mesenchymal cell model, where v-cyclin was shown to be needed for the KSHV induced transformation and post-confluent growth (Jones et al., 2014). v-cyclin was recently also shown to counteract the G1 cell cycle arrest caused by vFLIP and NF-κB hyperactivation (Zhi et al., 2014). In addition, transgenic mouse studies have shown that v-cyclin can act as an oncogene in the lymphocyte compartment, and this could be enhanced by p53 deficiency (Verschuren et al., 2004a, Verschuren et al., 2002).

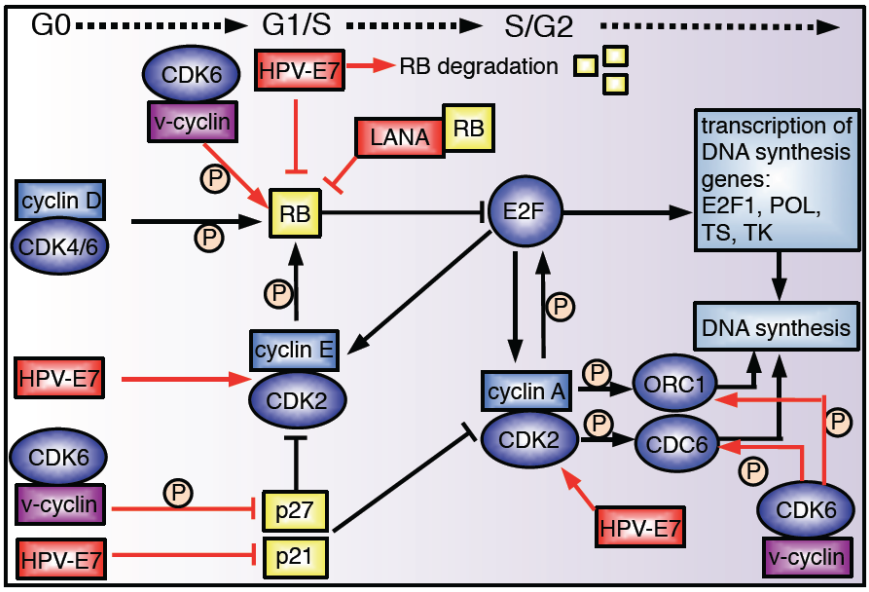

Figure 6. Regulation of the cell cycle by KSHV and HPV.

KSHV can regulate the G1-S cell cycle transition by encoding a homolog of cyclin D, v-cyclin, which together with its kinase partner CDK6 is able to phosphorylate several substrates such as pRb. pRb phosphorylation by v-cyclin-CDK6 abolishes E2F inhibition by pRb and activates transcription of S-phase genes. Furthermore, v-cyclin-CDK6 can phosphorylate the cyclinE-CDK2 inhibitor p27Kip1, as well as S-phase substrates, including ORC1 and CDC6. KSHV encoded LANA can bind pRb, and thereby inhibit its function. All these changes promote G1-S transition of the cell cycle. Highlighting the conserved nature of cell cycle regulation by human tumor viruses, the HPV E7 protein is able to induce S-phase entry via several mechanisms, including pRb degradation and inactivation of cell cycle inhibitors. Ⓟ=phosphorylation, (modified from (Boshoff and Weiss, 2002)).

3.1.1.2 vFLIP

Viral FLICE inhibitory protein (vFLIP) is a homolog of caspase 8 promodomain, and contains two death effector domains (DED) (Liu et al., 2002). Cellular and viral FLIPs block death receptor mediated apoptosis by preventing recruitment of caspase 8 to the death inducing signaling complex. However, KSHV vFLIP seems not to be able to inhibit Fas-mediated apoptosis *in vivo* (Chugh et al., 2005) but has unique function to inhibit apoptosis by inducing NF-κB. vFLIP binds to the IKK, which phosphorylates NF-κB inhibitor IκB leading to its degradation and finally releases the transcription factor dimer p50-p65 to bind DNA (Liu et al., 2002). In PEL cells, vFLIP mediated constitutional activation of NF-κB is essential for their survival and proliferation (Guasparri et al., 2004, Keller et al., 2000), and has been shown to require TNF receptor-associated factors TRAF2 and TRAF3 (Guasparri et al., 2006). vFLIP has also been shown to protect ECs from superoxide-induced cell death by upregulating manganese superoxide dismutase in NF-κB dependent manner (Thurau et al., 2009). In addition to NF-κB, vFLIP mediated increased survival has been linked to its ability to suppress autophagy by preventing Atg3 from binding and processing LC3 (Lee et al., 2009). Several models have been developed to study the vFLIP *in vivo*. vFLIP expressed under H2Kb promoter and immunoglobulin heavy chain (Eμ) enhancer in mice leads to NF-κB activation both by the canonical and alternate pathways (Chugh et al., 2005). These mice show increased proliferation of the splenocytes and increased incidence of lymphoma (Chugh et al., 2005). In a more sophisticated B-cell mouse model, Ballon et al. showed that vFLIP expression in the germinal center B-cells leads to formation of multicentric Castleman's disease like phenotype (Ballon et al., 2011). In ECs, vFLIP expression has been linked to the spindle morphology of the KSHV infected cells via NF-κB activity (Grossmann et al., 2006, Alkharsah et al., 2011). Furthermore, vFLIP has been shown to upregulate the expression of pro-inflammatory cytokines, chemokines, and interferon-responsive genes (Sakakibara et al., 2009, Thurau et al., 2009), and its depletion from the whole virus leads to reversion of Stat1-responsive gene activation and spindle cell morphology (Sakakibara et al., 2009).

3.1.1.3 vGPCR

An example of a KSHV oncogene expressed in the lytic phase is viral GPCR, which is a transmembrane protein sharing homology with the cellular IL-8 receptor (Cesarman et al., 1996). It can be activated by chemokines belonging to the CXC and CC families, but it is also constitutively active autonomous from the ligand binding (Arvanitakis et al., 1997, Bais et al., 1998, Gershengorn et al., 1998). vGPCR increases cell survival by inducing MAPK, PIK3/Akt/mTOR, and NF-κB pathways. Downstream of these pathways, vGPCR expression upregulates several transcription factors, including activator protein 1 (AP1), NFAT, NF-κB, HIF-1α, and cAMP-responsive element binding protein (CREB), leading to expression of VEGF and several pro-inflammatory cytokines and chemokines (Montaner, 2007). In ECs, this leads to increased proliferation and angiogenic potential *in vitro* (Bais et al., 2003, Montaner et al., 2001, Sodhi et al., 2006). In mouse models, transgenic vGPCR expression under an endothelial cell promoter has led to formation of angiogenic tumors dependent on AKT and mTOR (Montaner et al., 2003, Sodhi et al., 2006, Sodhi et al., 2004b). Furthermore, siRNA mediated depletion of vGPCR from the

whole virus led to decreased tumorigenic and angiogenic potential *in vivo* (Mutlu et al., 2007).

3.1.2 Kaposi's sarcoma

KS is divided into four clinico-epidemiological forms depending on the affected population: classic KS, endemic (African) KS, AIDS associated (epidemic) KS, and iatrogenic KS (Antman and Chang 2000). The classical KS described by Moritz Kaposi is manifested in the lower extremities of elderly Mediterranean men and usually has indolent natural progression. The endemic KS is found in equatorial Africa and shows a more aggressive behavior. The AIDS associated KS is usually very aggressive and is associated with advanced HIV infection usually accompanied by low CD4+ T-cell counts. The use of highly active antiretroviral therapy (HAART) has decreased the prevalence of AIDS-associated KS, but some cases seem refractory for the HAART treatment, and some cases have been described in patients with low HIV copy numbers and high CD4+ T-cell counts (La Ferla et al., 2013). The iatrogenic KS is seen in immunocompromised patients mostly after organ transplantations. KSHV infection is required for development of all forms of KS, and KSHV seroprevalence geographically correlates with the amount of KS cases, being especially high in Africa and in the Mediterranean region. In Finland, KSHV seroprevalence is low (estimated to be 1%), and the incidence of KS is 0.1-0.2 per 100 000 person-years in 1963-2010, age-adjusted to the World Standard Population (Aavikko, 2014, http://www.cancer.fi/syoparekisteri/). As not all KSHV infected individuals develop KS, additional factors are needed for the development of the disease. These include compromised immune system, other infections such as HIV and malaria (Conant et al., 2013), and local inflammatory milieu. However, the detailed mechanisms of the association/connection of KSHV and other factors still need further studies.

KS manifests as red to purple nodules or plaques on the skin. Later in its clinical progression, it can also affect mucosa of the mouth and visceral organs. The clinical staging is based on the amount of the lesions, and the extent of the spread to these different body sites. The individual lesions are thought to progress from early, flat patch lesions into raised plaques, and finally form tumor nodules. Histologically, the patch lesions consist of abnormal vessels, sparse chronic inflammatory cells, extravasated red blood cells and hemosiderin-loaded macrophages (Radu and Pantanowitz, 2013). In the plaque lesions, both the vessels and spindle cells are proliferating. The main component of the tumor nodules is the KSHV infected tumor cells, called spindle cells because of their morphology. In addition, the nodules exhibit a vast stromal component, comprising of chronic inflammatory cells and abundant hemosiderin-loaded macrophages (Radu and Pantanowitz, 2013). The spindle cells express markers of blood (CD31, CD34) and lymphatic (podoplanin, LYVE-1, VEGFR-3, Prox-1) endothelial cell lineages (Dupin et al., 1999, Kaaya et al., 1995), as well as markers of smooth muscle cells and fibroblasts (Kaaya et al., 1995, Sturzl et al., 1995, Weich et al., 1991), so the exact origin of these cells is not known. However, the prevailing view suggests of an endothelial origin (Ganem, 2010). Supporting this, especially the LECs are prone to KSHV infection *in vitro* and can be used as tools to model the infection cascade *ex vivo* (McAllister and Moses,

2007, Aguilar et al., 2012). Furthermore, it has been shown that KSHV infection can lead to transdifferentiation of BECs to LECs and vice versa, explaining the diversity of endothelial markers seen in the KS tumors (Carroll et al., 2004, Hong et al., 2004, Wang et al., 2004).

The mechanisms by which KSHV is causing KS are not fully understood despite extensive research. It is known that the KSHV genome contains multiple possible oncogenes and that viral genes can also affect cellular tumor suppressors (Jarviluoma and Ojala, 2006) (Figure 5, see above). However, much of the data comes from models of primary effusion lymphoma rather than KS, or are studies based on expression of one gene rather than in the context of the whole virus. As the isolated KS tumor cells lose the episomal genomes of KSHV upon culture (Grundhoff and Ganem, 2004), they cannot be used as models of KS tumor cells. Studying the development of KS by de novo KSHV infection has also proven difficult, as KSHV infection per se does not lead to transformation in cell models. In fact, the ECs respond to KSHV infection initially rather by a growth arrest and senescence than increased proliferation (Koopal et al., 2007, Lagunoff et al., 2002). However, this might be time dependent, as Wang and Damania showed that KSHV induced the PI3K/Akt/mTOR pathway and increased the survival and angiogenic potential of the human umbilical endothelial cells (HUVEC) after several weeks of culture when the culture was under selection for infected cells (Wang and Damania, 2008). This is supported by data showing that mTOR inhibitor rapamycin has an impact in treating KS both in the clinic and in a mouse model (Roy et al., 2013, Nichols et al., 2011). In addition, KSHV has been reported to activate the Notch pathway linked to tumorigenesis, angiogenesis, and differentiation in LECs via vFLIP and vGPCR mediated activation of Notch ligands Jag1 and Dll4. This was further shown to lead to downregulation of cell cycle components in the neighboring uninfected cell, giving the infected cells a growth benefit (Emuss et al., 2009).

Modeling the role of KSHV infection in the development of KS in mouse models has been challenging as well, since KSHV does not easily infect rodent cells. However, recently several attempts have been successful. Mutlu et. al showed that transfecting KSHV bacterial artificial chromosome (KSHVBAC36) into mouse bone marrow endothelial precursor cells can transform them and lead to KS like tumor formation in mice. In this model, siRNA suppression of vGPCR leads to reduced angiogenesis and tumorigenesis (Mutlu et al., 2007). In addition, it has been shown that KSHV can transform rat mesenchymal stem cells and lead to tumor formation *in vivo* (Jones et al., 2012). To study the role of individual genes in this system, Jones et al. deleted viral genes individually. Using this approach they were able to show that v-cyclin expression was needed to drive oncogenesis and override contact inhibition (Jones et al., 2014). Further suggesting that rodent progenitor cells can be infected, Ashlock et al. showed that mouse bone marrow derived progenitor cells could be infected by recombinant KSHV, and these cells could give rise to tumors in mice (Ashlock et al., 2014). Moreover, humanized NOD/SCID/IL2γ mice were shown to be susceptible for KSHV infection through natural infection routes, possibly providing a model system to study KSHV pathogenesis in future (Wang et al., 2014).

3.1.3 Primary effusion lymphoma (PEL)

KSHV associated PEL is a very rare but aggressive form of HIV-associated non-Hodgkin's lymphoma that accounts for 4% of the cases. The median survival after diagnosis is only six to nine months (Boulanger et al., 2005). PEL is almost exclusively detected in KSHV positive HIV infected individuals, and the lymphoma cells may be co-infected with EBV (Cesarman et al., 1995a, Cesarman et al., 1996, Nador et al., 1996). PELs are characterized by proliferation of lymphoid cells in pleural, peritoneal, and pericardial effusions, and they typically do not form a solid tumor mass. The lymphoma cells exhibit "null" lymphocyte phenotype as they express the common lymphocyte marker CD45, but not markers for B-cells (CD19, CD20, CD79a or surface immunoglobulins) or T-cells (CD3, CD4, CD8). However, they do express markers of plasma cell differentiation (CD138) and activated lymphocytes (CD30, CD38, CD71). Immunogenotypic studies usually show immunoglobulin gene rearrangements and somatic hypermutations, suggesting a post-germinal center B-cell origin (Klein et al., 2003). This is supported by *in vitro* observations, showing that even though B-cells are not readily infectable by KSHV, successful B-cell infection drives them towards plasmablast differentiation (Hassman et al., 2011). In contrast to KS cells, PEL cells retain viral genomes in culture, and tumor cell lines can be obtained (Arvanitakis et al., 1996, Cesarman et al., 1995b). Moreover, when injected subcutaneously or intraperitoneally into mice, the PEL cells form tumors (Dai et al., 2014).

Many viral factors and cellular signaling pathways have been linked to the growth and survival of the PEL cells. PEL cells have been shown to be dependent on vFLIP induced NF-κB activation *in vitro* and *in vivo* (Guasparri et al., 2004, Keller et al., 2000). In addition, there have been reports showing that PEL cells are dependent on Notch signaling, as the gamma secretase inhibitor DAPT treatment leads to cell cycle arrest and apoptosis of PEL cells *in vitro* and *in vivo* (Lan et al., 2009). One mechanism by which KSHV is activating Notch in PEL cells is LANA mediated stabilization of NICD1 (Lan et al., 2006, Lan et al., 2007). Furthermore, several viral and cellular cytokines, including viral IL-6, IL-6, IL-10, stromal cell-derived factor 1, and VEGF have been shown to be important for the PEL cell survival and growth (Gasperini et al., 2008).

3.2 MELANOMA

Melanoma is a cancer of melanocytes, the pigment producing cells of the skin and mucosa. Clinically it appears as pigmented macules, and should be suspected if changes in size, color, or symmetry appear in pre-existing melanocytic lesions or new lesions arise at older age (Muller, 2014). Melanoma comprises four percent of all cancers, and approximately 1200 cases are diagnosed yearly in Finland (http://www.cancer.fi/syoparekisteri/). The incidence of melanoma is rising in Caucasian populations and most steeply in young women, possibly because of increased UV radiation to previously protected areas of the body (Rastrelli et al., 2014). Due to its poor prognosis after metastatic spread, it is the most notorious of all skin cancers, even though

the combined 5-year survival rate was 84% of male and 89% of female patients in Finland in 2012 (http://www.cancer.fi/syoparekisteri/). The risk factors of melanoma include both genetic, most notably fair skin, and environmental factors such as extensive UVB/A radiation. Even though the sun exposure is considered the main risk factor for the onset of melanoma, its role is complex. The most commonly affected sites in light skinned persons are in fact the areas that are exposed to sun intermittently, not chronically, and melanoma can arise also in mucosal membranes and acral sites, which are not exposed to sun (Whiteman et al., 2001).

Melanomas are divided into clinicopathological subtypes, the most common types being lentigo maligna melanoma, superficially spreading melanoma, and nodular melanoma (Muller, 2014). These types follow the widely accepted concept of radial and vertical growth phases in melanoma progression. In radial growth phase, the tumor cells are found intraepidermally as flat circles of pigmented cells, whereas the vertical growth phase is characterized by tumor cells infiltrating the dermis (Chin, 2003). Angiogenesis and expression of vascular growth factors are also associated with the progression to the vertical growth phase (Braeuer et al., 2011). Superficially spreading melanoma shows first radial growth phase and only after a long period of time shows invasion into deeper tissues. In contrast, nodular melanoma starts the vertical growth phase early in its history, and thus has a poorer prognosis.

3.2.1 Melanoma initiation

The most common genetic alteration in melanocytes leading to their transformation is an activating BRAF mutation caused by an amino acid substitution from valine (V) to glutamate (E) at codon 600 (refered as V600E) (Davies et al., 2002, Curtin et al., 2005). This mutation can be seen in 50% of the melanoma patients, and leads to activation of MAPK signaling. Mechanistically, the autonomous activation in BRAF leads to phosphorylation of MEK and ERK, and finally activation of cyclin D1-CDK4/6 complex and progression of the cell cycle. In cases where BRAF is wild type, its upstream regulator N-RAS is commonly (20% of cutaneous melanomas) mutated, leading to the activation of the same pathway. Especially in mucous melanomas, the receptor tyrosine kinase c-KIT, which is affecting the same downstream pathways, is often mutated. In cases where these regulators are not affected, the downstream effector cyclin D1 gene *CCND1* and its kinase partner CDK4 gene regions are commonly amplified (Curtin et al., 2005). The cell cycle inhibitor p16 gene CDKN2A locus is the most commonly deleted region (50%) in melanoma further highlighting the importance of this pathway (Curtin et al., 2005). Moreover, the PI3K pathway inhibitor PTEN is commonly deleted in BRAF mutant but not in N-RAS mutant melanomas, suggesting that the MAPK and PI3K work in parallel downstream of RAS to promote melanoma formation.

3.2.2 Melanoma progression

Even though the MAPK pathway is the major driver of melanoma growth, the pathways leading to tumor progression and metastasis are more widespread. This is important to

notice as virtually all deaths associated to melanoma are due to metastases. More than 95% of patients who initially have early stage disease are cured from melanoma by surgery, whereas the 5-year survival rate of patients with visceral metastases is less than 10% (Balch et al., 2009). Treatments of advanced melanoma that have been concentrating on inhibition of the MAPK pathway first seemed beneficial, but patients soon developed resistance to them. The most commonly used targeted therapy has been veramurafenib, a BRAF inhibitor, which has shown good response for 6-9 months (McArthur et al., 2014), but then the BRAF inhibition has been bypassed by activated RAS. Combination of BRAF inhibitors with MEK inhibitors have led to a prolonged progression free survival from 6.3 months to 11 months, but has as well been curative only in very few patients (Larkin et al., 2014). Furthermore, in some contexts these inhibitors might even be promoting the metastasis. It was recently shown that BRAF/MEK inhibitors can increase the invasive potential of 20% of the melanoma cell lines by regulating STAT3 (Vultur et al., 2014). Thus, understanding the mechanisms of melanoma metastasis is crucial for developing better treatments.

The best-studied prognostic factors considering the primary tumor are tumor thickness and ulceration, highlighting the importance of the transition from the radial to vertical growth phase. It is accompanied by molecular events including the loss of adhesive molecules and increased expression of proteases such as MMPs, leading to increased invasiveness (Villanueva and Herlyn, 2008, Moro et al., 2014). This might be due to deregulated expression of multiple transcription factors, including AP-2a, CREB, and NF-κB (Mobley et al., 2012). Other mechanisms that control the tumor progression are the pathways conducting tumor cell differentiation and plasticity, which in turn enable the tumors to interact and adapt to the tumor microenvironment (Hendrix et al., 2003). It has recently been shown that hypoxia can induce phenotypic plasticity in melanoma via activation of the tyrosine kinase receptor ROR2 (O'Connell et al., 2013). Furthermore, aggressive undifferentiated melanoma cells express Nodal, an embryogenic morphogene, which has a key role in regulating melanoma cell plasticity by mediating SMAD2 and SMAD3 phosphorylation (Hendrix et al., 2007, Topczewska et al., 2006). The Nodal phenotypes have been shown to be regulated by Notch4 expression, suggesting that these two embryonal pathways are interacting and together regulate the melanoma cell plasticity (Hardy et al., 2010). The other Notch receptors have also been shown to be involved in the melanoma progression, as Notch1 has been shown to act as an effector of AKT signaling (Bedogni et al., 2008), whereas Notch2 induces invasion in uveal melanoma (Asnaghi et al., 2012), and Notch3 has been shown to be upregulated by EC interaction (Howard et al., 2013).

Lymphatic involvement has significance as a prognostic factor of melanoma. If only microscopic lymph node metastases are found (stage TxN1aM0), the 5-year survival is approximately 70%, while in the group of abundant lymph node metastasis (stage TxN3N0) it decreases to 30% (Balch et al., 2009). In addition, the primary tumor lymphatic vessel density, the relative area of lymphatic vessel invasion and the VEGF-C expression levels correlate with the metastases and poor prognosis (Dadras et al., 2005, Dadras et al., 2003). However, it is inadequately understood how the lymphatic

involvement regulates the melanoma distant metastasis and prognosis, as the removal of the metastatic lymph nodes has no survival benefit (Alitalo and Detmar, 2012, Sleeman et al., 2011). It is known that melanoma can directly, or indirectly through the immune cells, lead to VEGF-C expression in the tumors, and induce lymphangiogenesis (Peppicelli et al., 2014, Peppicelli et al., 2013). In addition, the chemokine ligand-receptor pairs have been shown to be important in the chemotaxis of the tumor cells towards the lymphatics (Cabioglu et al., 2005, Shields et al., 2007, Das et al., 2013). The specific tumor homing to different tissues, including skin and soft tissues followed by lung, liver, brain, bone, and basically all other organs, has been suggested to be also due to chemokine ligand-receptor binding (Kakinuma and Hwang, 2006). Examples include the CCR7/CCL21 axis, which has been linked to lymph node metastasis, whereas the CXCR4/ CXCL12 axis has been shown to be involved in liver, lung, and bone marrow metastasis (Raffaghello et al., 2009).

AIMS OF THE STUDY

Tumor cells in both KSHV associated malignancies and melanoma exhibit features of cellular plasticity. Even though Kaposi's sarcoma (KS) is thought to be of endothelial origin, KS tumor cells express markers of many lineages, including blood and lymphatic vasculature as well as smooth muscle cells and fibroblasts. In addition, the KSHV associated primary effusion lymphoma (PEL) has an undifferentiated plasma cell phenotype. Melanoma cells, on the other hand, have been shown to be able to undergo vascular mimicry, as well as to interact with the blood and lymphatic endothelial cells during cancer progression. The aim of this study is to understand what changes in the differentiation state or cellular reprogramming are involved in the pathogenesis of KS, PEL, and melanoma, and what the potential molecular mechanisms behind these events are. Specifically, the aims are:

1. To study KSHV oncogene v-cyclin mediated oncogenesis mechanisms in the lymphocyte compartment *in vivo*

2. To untangle the mechanism behind diverse expression of lineage markers in KS tumor cells

3. To study if the lymphatic endothelial cell - tumor cell interactions lead to reprogramming and contribute to tumor progression in melanoma

MATERIALS AND METHODS

1. Mouse experiments (I, II, IV)

All mouse lines used in the studies and their references are described in Table 1. Briefly, v-cyclin construct containing double flag-tag was expressed under the immunoglobulin heavy chain promoter/enhancer Eμ, which directs the expression to the lymphocyte compartment. These mice were bred into the outbred ICR (CD1) mouse background for at least five generations to generate the ICR- Eμ-v-cyclin line, or backcrossed to the inbred C57BL6 background for at least five generations to generate the BL6- Eμ-v-cyclin line. To test the tumorigenicity and metastatic capacity of the melanoma cells, the control Bowes and WM852 cells, as well as the same melanoma cells primed with primary LECs, were injected subcutaneously (s.c.) into immunocompromised C.B-17/IcrHanTMHSD-Prkdc Scid (SCID) mice. The melanoma cells contained a double eGFP-luciferase reporter, enabling the *in vivo* imaging of the luciferase signal (Caliper IVIS Kinetic System) from the tumors and metastases. All mouse experiments were approved by the Finnish National Animal Experiment Board (license numbers: ESLH-2005-03350/Ym-23, ESLH-2006-04075/Ym-23, ESLH-2009-02139/Ym-23, ESAVI/434/04.10.03/2012).

Table 1. Mice used in this study.

background	description	source/reference	used in
C.B-17/IcrHanTMHSD-Prkdc Scid	mice having severe combined immunodeficiency, i.e., lacking functional T- and B-cells	Harlan Laboratories	IV
C57BL6	inbred mouse line	Harlan Laboratories	I
C57BL6-Eμ-v-cyclin	v-cyclin transgene under Eμ promoter/enhancer in C57BL6 mouse background	Publication I	I
CBA/C57BL6-Eμ-v-cyclin	v-cyclin transgene under Eμ promoter/enhancer in mixed mouse background	(Verschuren et al., 2004a, Verschuren et al., 2002)	I
Eμ-Myc	c-Myc transgene under Eμ promoter/enhancer	(Adams et al., 1985)	I
ICR (CD1)	outbred mouse stock	Harlan Laboratories	I, II
ICR-Eμ-v-cyclin	v-cyclin transgene under Eμ promoter/enhancer bred to ICR strain	Publication I	I, II

2. Cell lines (I, II, III, IV)

The cell lines used are listed in Table 2, and the culture conditions are described in the given references. In short, for the lymphoma cell lines generated in publication I, lymphoma tissues were disaggregated by pressing through a 70-μm nylon mesh cell strainer (BD Falcon) to obtain a single cell suspension. The isolated lymphoma cells were

cultured in lymphoma cell media (Schmitt et al., 2002) at density between $2x10^5$ to 10^7 cells/ml and grown for at least three passages before further analysis.

Table 2. Cell lines used in this study.

cell line	description	source/reference	used in
BC-3	PEL cell line	NIH AIDS Reagent Program	II
BCBL-1	PEL cell line	NIH AIDS Reagent Program	I, III
BEC	primary blood endothelial cells	Promocell	IV
Bowes	melanoma cell line (superficially spreading)	Dr. Kaisa Lehti	IV
Eμ-myc thymus lymphoma	cell line from Eμ-Myc thymus lymphoma	Dr. Anna Cvrljevic	I
HEK293	Human Embryonic Kidney 293 cells	Biomedicum Functional Genomics Unit	I, II, III
HEK293A	HEK293 cells, adherent clone	Dr. Markus Vähä-Koskela	I
HEK293-FlipIn	HEK293 cell line designed for rapid generation of stable cell lines	Invitrogen	I
LEC	primary lymphatic endothelial cells from juvenile foreskin	Promocell	III, IV
	primary lymphatic endothelial cells from adult skin	Lonza	IV
Phoenix Ampho	second generation retrovirus producer line		III, IV
U2OS	human osteosarcoma cell line		III
v-cyc1 thymus lymphoma	cell line from Eμ-v-cyclin mouse thymus lymphoma	Publication I	I
v-cyc1 spleen lymphoma	cell line from Eμ-v-cyclin mouse spleen lymphoma	Publication I	I
v-cyc1 lymph node lymphoma	cell line from Eμ-v-cyclin mouse lymph node lymphoma	Publication I	I
v-cyc2 thymus lymphoma	cell line from Eμ-v-cyclin mouse thymus lymphoma	Publication I	I
v-cyc2 spleen lymphoma	cell line from Eμ-v-cyclin mouse spleen lymphoma	Publication I	I
v-cyc3 thymus lymphoma	cell line from Eμ-v-cyclin mouse thymus lymphoma	Publication I	I
WEHI-3B	mouse B-cell lymphoma line	Dr. Anna Cvrljevic	I
WM852	melanoma cell line (skin metastasis)	Dr. Kaisa Lehti	IV

3. Cell culture models (III, IV)

3.1 2D co-culture of melanoma and endothelial cells (ECs)

For physical co-culture, melanoma and LECs were seeded together on fibronectin (Sigma) or gelatin pre-coated cell culture plates in 1:1.5-1:3 ratio in endothelial cell culture media (EGM-2, Lonza), whereas similarly treated single-cultured cells served as controls. After 48-72 h, the cells were either fixed for stainings or separated. For cell separations, the melanoma cells were pre-labeled with 1mg/ml fluidMAG-DX (Chemicell) magnetic beads

before the co-culture. The actual separations were done using the MidiMACS separator and LS column (both from Miltenyi Biotec), and collecting both the non-labeled (LEC) and labeled (melanoma) fractions for further use for immunofluorescent stainings, RNA lysates, or functional assays. To study the adhesion properties after the co-culture, the co-cultured or single-cultured cells were seeded on top of a confluent layer of LECs, and allowed to interact for 12 h before fixation for stainings. To study the supernatant mediated effects, conditioned media experiments were used. Briefly, the LECs were cultured in the endothelial media for 24-48 h before collection of the supernatant, which was deprived for cells by filtering. The melanoma cells were incubated with the conditioned media for 24-72 h before fixation for stainings, or collection for RNA or protein lysates.

3.2 2D cell migration assay

Cell migration in 2D was studied by using wound healing assay. Briefly, $5x10^4$ melanoma or LEC cells in 70 μl of endothelial media were plated homo- or heterotypically on Ibidi inserts on 12-well plates coated with fibronectin, and let to attach for 12 h. Following the removal of the insert, the plates were immediately transferred to Cell IQ automated phase contrast microscope (CM Technologies; time point 0 h), and the wells were followed for 48 h at 30 min intervals, during which the wound was fully closed in all samples.

3.3 Fibrin embedded cultures

Monolayers of BECs, LECs, or KSHV infected LECs (K-LEC) were seeded onto 0.5% agarose pre-coated, non-adherent round-bottom 96-well plates at 4000 cells per well. After 16-24 h incubation at 37°C, the spontaneously formed spheroids were harvested and embedded into the fibrin gel consisting of plasminogen-free fibrinogen (final concentration 3-5 mg/ml; Calbiochem) and thrombin (final concentration 2 U/ml; Sigma) in Hank's Balanced Salt Solution supplemented with aprotinin (200 μg/ml; Sigma). When co-cultured with the melanoma cells, the EC spheroids were mixed with 5000-10000 melanoma cells prior to adding the fibrinogen and the enzymes. For fibrin invasion assay, single-cultured or 2D co-cultured melanomas were embedded at density of 5000 cells/gel. After complete gelling, culture medium containing 50-100 μg/ml aprotinin was added on top of the gels to prevent gel dissolving, and they were followed for 2–5 days.

3.4 Quantification of spheroid sprouting

Quantification of spheroid sprouting was performed from the phase contrast or fluorescent images. From the phase contrast images, the sprouts and the spheroid body boundaries were depicted using the Inkscape software [http://www.inkscape.org]. The vector graphic data was first rendered with Inkscape, and then analyzed in a pipeline generated using the Anduril framework (Ovaska et al., 2010). This pipeline searches the sprout lines and counts them, gives the total length of the sprouts and the spheroid body area. Sprouting is defined as the total length of the sprouts normalized to the spheroid body area.

4. Virus production and infections (I, II, III, IV)

The viruses used are listed in Table 3, and the details of the production and infections are described in the given references. Briefly, the wild type KSHV was produced in BCBL-1 cells, and the recombinant KSHV (rKSHV.219) in the Vero cells by stimulating them with known chemical inducers of lytic reactivation (12-O-Tetradecanoyl-phorbol-13-acetate (TPA) for BCBL-1, and sodium buturate (NaB) combined with baculovirus expressing KSHV replication and transcription activator (RTA) for Vero). The concentrated virus in basal endothelial media supplemented with polybrene was used to spin-infect LECs at multiplicity of infection (MOI) 1, and the efficacy of infection was determined by LANA staining. The various overexpression and shRNA vectors used to produce lenti- and retroviruses are described in Tables 3 and 4. The VSV-pseudotyped lentiviruses were produced from HEK293FT cells transfected with the given vector and the packaging plasmids (pLP1, pLP2, pLP/VSVG), and concentrated by ultracentrifugation. The retroviruses were produced in Phoenix Ampho cells containing the retroviral packaging plasmids by transfecting only the desired construct. The lenti- and retroviruses were used as one time concentrated viral supernatant to spin-transduce HEK293A, HEK293-FlipIn, LEC, Bowes, and WM852 cells. The samples for further analysis were collected 48-72 h after transductions.

Table 3. Viruses used in this study.

virus	description	reference/source	used in
GFP-luc retrovirus	retrovirus containing fusion eGFP-luciferase reporter	Dr. Kaisa Lehti	IV
KSHV	wild type KSHV produced from BCBL-1 cells	Publication III	III
LANA lentivirus	overexpression construct in pLenti6/V5/DEST lentiviral vector	(Sarek et al., 2006)	III
MT1-MMP retrovirus	overexpression construct in pMX-GFP retroviral vector	(Ory et al., 1996)	III
rKSHV.219	recombinant KSHV containing the KSHV genome and expressing GFP under the cellular EF-1alpha promoter to detect all infected cells and RFP under the viral PAN promoter to detect cells in lytic infection phase	(Vieira and O'Hearn, 2004)	III
sh-CDK6 lentivirus	shRNA against human CDK6 in pDSL_hpUGIH backbone	(Koopal et al., 2007)	II
sh-CDK6_1 lentivirus	shRNA against human CDK6 in pLKO.1 vector backbone	Biomedicum Functional Genomics Unit	I
sh-CDK6_2 lentivirus	shRNA against human CDK6 in pLKO.1 vector backbone	Biomedicum Functional Genomics Unit	I
sh-scr lentivirus	scrambled shRNA in pDSL_hpUGIH vector backbone	(Koopal et al., 2007)	II
sh-scr lentivirus	scrambled shRNA in pLKO.1 vector backbone	Biomedicum Functional Genomics Unit	I

v-cyclin lentivirus	overexpression construct in pBMN-IRES-GFP retroviral vector	(Koopal et al., 2007)	III
vFLIP lentivirus	overexpression construct in pSIN-MCS lentiviral vector	(Lagos et al., 2007)	III
vGPCR lentivirus	overexpression construct in pSIN-MCS lentiviral vector	(Lagos et al., 2007)	III

5. RNAi (III)

For RNA interference experiments, cells were transfected using siRNAs listed in Table 4 with Oligofectamine (Invitrogen) according to manufacturer's instructions. Spheroids were prepared one day post siRNA transfection.

Table 4. shRNA/siRNA constructs used in this study.

shRNA/siRNA	sequence/reference no	source/reference	used in
CDK6 shRNA	5′-GATCCGAGTAGTGCATCGCGATCTTTCAAGAGAAGATCGCGATGCACTACTCGGTTTTTTGA-3′	(Koopal et al., 2007)	II
CDK6 shRNA_1	TRCN0000039747	Biomedicum Functional Genomics Unit	I
CDK6 shRNA_2	TRCN0000194893	Biomedicum Functional Genomics Unit	I
ctrl siRNA for vFLIP	5'-AAGCGCGCUUUGUAGGAUUCG-3'	(An et al., 2003, Guasparri et al., 2004)	III
ctrl siRNA for vGPCR	5'-AGTACCGACATCCGACCAA -3'	(Bottero et al., 2009)	III
MT1-MMP siRNA1	5'-CAGCGATGAAGTCTTCACTTA-3'	Qiagen	III
MT1-MMP siRNA2	5'-TGGCGGGTGAGGAATAACCAA-3'	Qiagen	III
non-target control siRNA	ND	Qiagen	III
non-target control siRNA	ND	Dharmacon	III
PDGFRA siRNA	siGENOME SMARTpool	Dharmacon	III
PDGFRB siRNA	siGENOME SMARTpool	Dharmacon	III
scr shRNA	5′-ATCCGTTCTCCGAACGTGTCACGTTTCAAGAGAACGTGACACGTTCGGAGAATTTTTTGGAAA-3′	(Koopal et al., 2007)	II
scr shRNA	SHC005	Biomedicum Functional Genomics Unit	I
vFLIP siRNA 1	5'-AACGUGUUCAUACCUCAACCC-3'	(An et al., 2003, Guasparri et al., 2004)	III
vFLIP siRNA 2	5'-AAGUGGUAUUGUUCCUCCUAA-3'	(An et al., 2003, Guasparri et al., 2004)	III
vGPCR siRNA1	5'-GAACGTTGGAATACTCTCT-3'	(Bottero et al., 2009)	III
vGPCR siRNA2	5'-GGTACTGACATCCGCTGCA-3'	(Bottero et al., 2009)	III

6. Inhibitor and ligand stimulation assays (I, III, IV)

The inhibitors and ligands used are described in Table 5. Briefly, mouse lymphoma cells and PEL cells at a starting density of $2x10^5$ cells/ml were incubated for 72 hours with DAPT (10 μM; Sigma) or PD0332991 (0.5-1 μM; Adooq Bioscience) or corresponding vehicle control (EtOH/DMSO). The number of live and dead cells were determined by trypan blue exclusion and counting with a TC10 Automated cell counter (Bio-Rad) at 0 h, 24 h, 48 h and 72 h after adding the inhibitor. Cell pellets for analysis by real time quantitative PCR were collected at 24 h. For spheroid assays, the inhibitors were added into the medium after the spheroids were embedded into the fibrin gel and followed for 2–4 days prior fixation or collection for RNA lysates. Human VEGF-A and VEGF-C were included in the medium 1 day before spheroid preparation, during spheroid formation, and for 2 days in 3D. In IV, the LEC-WM852 single- or co-cultures were treated with DAPT (10 μM; Sigma), AMD3100 (10 μM; Calbiochem), or their respective vehicle controls (DMSO/mock) for 48 hours prior the adhesion assay, during which the inhibitors were also kept on.

Table 5. Inhibitors and ligands used in this study.

Inhibitor /ligand	description	source/reference	used in
AMD3100	Inhibitor of CXCR4 ligand binding	Calbiochem	IV
cytochalasin D	inhibitor of actin polymerization	Sigma	III
DAPT	gamma-secretase inhibitor	Sigma	I, III, IV
DLL4-Fc	signal peptide and extracellular domain of human DLL4 fuced to human Fc fragment	Prof. Kari Alitalo	III
GM6001	broad-spectrum matrix metalloproteinase inhibitor	Calbiochem	III
nocodazole	inhibitor of microtubule polymerization	Sigma	III
PD0332991	CDK4/6 kinase inhibitor	Adooq Bioscience	I
PDGF-AA	ligand for PDGFR-α receptor	R&D systems	III
PDGF-BB	ligand for PDGFR-β receptor	R&D systems	III
rTGFB-1	recombinant TGF-β	R&D systems	III
SB431542	inhibitor of TGF-β superfamily type I activin receptor-like kinase (ALK) receptors	Sigma	III
SU16f	PDGFR-β inhibitor	Tocris Bioscience	III
TIMP-1	tissue inhibitor of metalloproteinases 1	R&D systems	III
TIMP-2	tissue inhibitor of metalloproteinases 2	R&D systems	III
VEGF-A	vascular endothelial growth factor A	R&D systems	III, IV
VEGF-C	vascular endothelial growth factor C	Prof. Kari Alitalo	III, IV

7. Immunofluorescence (IF) of 2D and 3D cultures (II, III, IV)

The 2D and 3D cultured cells were fixed with 4% paraformaldehyde for 15-60 min at room temperature, permeabilized with 0.1-0.3% Triton-X, and the 3D cultures were additionally treated with ice cold acetone-methanol for 1 min. Immunofluorescence was performed on fixed 2D or 3D cultures. The primary antibodies used are listed in Table 6. Stainings with omitted primary antibodies were used as negative controls. For the immunofluorescence detection, secondary antibodies labelled with fluorochromes Alexa Fluor 488, 594 and 647, Cy3, or Hoechst 33342 were used. The fluorescent images were aquired by using Zeiss epifluorescence microscopes, CellInsight automated epifluorescence microscope, or confocal imaging by Zeiss or Leica confocal imaging systems.

8. Immunofluorescence or immunohistochemistry (IHC) of tissue sections (I, II, IV)

After dissection, mouse tissues and tumors were fixed with 4% paraformaldehyde for 24 h at +4°C, embedded in paraffin and cut into 5 μm sections. H&E stainings were performed according to standard protocols. Before primary antibody incubation, tissue sections were treated for antigen retrieval with 10 mM citrate buffer (pH 6.0) or 0.25% trypsin solution. The primary antibodies used are listed in Table 6. Omission of the primary antibody, or isotype and concentration matched primary antibodies served as negative controls. In immunohistochemical stainings, signal was detected and amplified using the tyramide signal amplification (TSA) biotin system kit (Perkin Elmer) according to the manufacturer's instructions, and analyzed by Zeiss light microscopes. In fluorescent stainings, fluorochromes listed in section 7 were used for detection, and images were acquired by using Zeiss epifluorescence microscopes, or confocal imaging by Zeiss or Leica confocal imaging systems. The image analysis of the KS tissue stainings was done in a pipeline created in the Anduril framework (Ovaska et al., 2010). The permission to use KS tissue material from patients was given by the Helsinki University Central Hospital (HUCH) ethical committee (to Lauri Aaltonen, permission number: 408/13/03/03/2009).

9. Fluorescence-activated cell sorting (FACS) (I)

For FACS, $2x10^6$ of freshly isolated splenic and thymic lymphocytes were incubated for 1 h at +4°C with specific pre-conjugated antibodies listed in Table 6. After the surface marker labeling, lymphocytes were washed with 1% BSA in PBS and fixed with 0.01% paraformaldehyde for 15 min at +4°C, and washed twice. For Ki-67 stainings, the cells were permeabilized with 0.5% Tween 20 for 15 min before 1 h incubation at +4°C with the antibody. To determine the cell cycle profiles, lymphocytes were stained with propidium iodide (PI, Sigma) after fixation of the cells with 70% ethanol in -20°C. Labelled cells were acquired using a FACSAria II flow cytometer (BD Biosciences), and cell populations were analyzed by BD FACSDiVa Software v 6.1.

Table 6. Primary antibodies and stains used in this study.

antibody against/ stain	description	source/reference	used in
Annexin V	APC conjugated stain against apoptotic cells	BD Pharmingen	I: FACS
α-SMA	Cy3 conjugated mouse antibody, clone 1A4	Sigma	III, IV: IF
B220	rat antibody (RA3-6B2)	Southern Biotech	I: IHC
CD3	rabbit antibody (A0452)	Dako	I: IHC
CD3	PE-Cy7 conjugated primary antibody (145-2C11)	BD Pharmingen	I: FACS
CD4	PE conjugated primary antibody, (H129.9)	BD Pharmingen	I: FACS
CD8	FITC conjugated primary antibody (53-5.8)	BD Pharmingen	I: FACS
CD44	mouse antibody (sc-7297)	Santa Cruz	IV: IF
CDK2	rabbit antibody (sc-163)	Santa Cruz	I: IB, IP
CDK4	rabbit antibody (C22, sc-260)	Santa Cruz	II: IB
CDK6	mouse antibody (#MS-451)	Thermo Scientific	I, II: IB
CDK6	rabbit antibody (C21, sc-177)	Santa Cruz	I, II: IB, IP
Cyclin A	rabbit antibody (sc-596)	Santa Cruz	I: IB
Cyclin D3	rabbit antibody (sc-182)	Santa Cruz	I, II. IB
CXCR4	rabbit antibody	R&D system	IV: IF
fibronectin	rabbit antibody rabbit antibody	Prof. Antti Vaheri Sigma	III: IF IV: IHC
flag M2	mouse antibody (F1804)	Sigma	I, II: IB, IP
γ-tubulin	mouse antibody (GTU-88)	Sigma	I, II, III: IB
GFP	rabbit antibody	Dr. Giuseppe Balistreri	IV: IF
Ki-67	PerCP-Cy5.5 conjugated antibody (B56)	BD Pharmingen	I: FACS
LANA	rabbit antibody	Prof. Bala Chandran	III: IF
LANA	rat antibody	ABI Biotechnologies	III: IF
LYVE-1	rabbit antibody against human LYVE-1	Prof. Pirjo Laakkonen	III: IF
LYVE-1	rabbit antibody against mouse LYVE-1 (103-PA50)	Reliatec	IV: IHC
mouse IgG	(sc-2025)	Santa Cruz	III: IF
MT1-MMP	mouse antibody	Chemicon	III, IV: IF, IHC, IB
N-cadherin	rabbit antibody	BD Pharmingen	III: IF
NICD1	(ab8925)	Abcam	I: IB
NICD3	(sc-7424)	Santa Cruz	I: IB
Notch3	(N5038)	Sigma	I: IF
NPM	mouse antibody (32-5200)	Invitrogen	I: IB
NPM – phospho-T199	rabbit antibody (CST3541)	Cell Signaling	I: IB
ORF59	mouse antibody	Prof. Bala Chandran	III: IF
p65	rabbit antibody (sc-372)	Santa Cruz	II: IB, IF
p65 phospho-Ser536	rabbit antibody (CST3033)	Cell Signaling	II: IB, IF
PDGFR-α	mouse antibody	Santa Cruz	III: IB, IF
PDGFR-β	mouse antibody	Santa Cruz	III: IB, IF
PECAM-1	mouse antibody rat antibody	Dako BD Pharmingen	III, IV: IF IV: IHC

Phalloidin	stain for actin cytoskeleton, Alexa488 conjugated	Invitrogen	IV: IF
podoplanin	rabbit antibody	(Breiteneder-Geleff et al., 1999)	III: IF
Prox-1	goat antibody (AF2727)	R&D Systems	IV: IF
rabbit IgG	(sc-2027)	Santa Cruz	III: IF
rat IgG	(sc-2026)	Santa Cruz	III: IF
SP1	(PEP2, sc-59)	Santa Cruz	II: IB
transgelin		Abcam	III: IF
v-cyclin	rabbit antibody	(Sarek et al., 2006)	I, II: IB, IP
VE-cadherin	mouse antibody (555661)	BD Pharmingen	III, IV: IF
VE-cadherin	goat antibody (AF357)	R&D Systems	IV: IF
VEGFR3	mouse antibody (clone 9D9)	(Jussila et al., 1998)	III: IF
vimentin	mouse antibody	V9; Dako	III: IF
ZO-1	rabbit antibody	Zymed	III: IF

10. Subcellular fractionation, immunoprecipitations (IP), *in vitro* kinase reaction, and immunoblotting (IB) (I, II, III)

For immunoprecipitations, and their whole cell lysate controls, cells were lysed in an ELB lysis buffer (50 mM Hepes (pH 7.4), 150 mM NaCl; 50 mM HEPES, pH 7.4; 0.1% Igepal; 5 mM EDTA). Alternatively, the total cell lysates were prepared in Urea-Tris lysis buffer (UTB) (9 M Urea, 75 mM Tris-HCl, pH 7.5, 0.15 M 2-mercaptoethanol) or NET buffer (150 mM NaCl, 50 mM EDTA, 100 mM Triton-X100), and subcellular fractionations were done using series of lysis buffers as earlier described (Sarek et al., 2006). All lysis buffers were supplemented with complete proteinase inhibitor cocktail (Thermo Scientific), and phosphatase inhibitory cocktail when appropriate (PhosphoSTOP, Roche), and the lysates were homogenized by passing the cell lysate through a 23-gauge needle or sonication, and cleared by centrifugation. For immunodepletions or immunoprecipitations (IP) 300-1000 μg of protein were used per sample and incubated with antibodies listed in Table 6. *In vitro* kinase reaction was performed after IP by using GST-Rb and Histone H1 as substrates. Immunoprecipitated and total proteins (15-75 μg) were subjected to SDS-PAGE and transferred to nitrocellulose membranes. For immunoblotting (IB), membranes were probed with antibodies described in Table 6.

11. Real time quantitative PCR (qRT-PCR) (I, III, IV)

Total RNA was extracted using the RNeasy mini kit (Qiagen) or the NucleoSpin RNA II kit (Macherey Nagel). Transcript levels were measured by qRT-PCR using Taqman Gene Expression Assays (Applied Biosystems) with the FAM-labeled primers, or unlabeled primers using the SYBR Green PCR mix (Fermentas) in the StepOnePlus Real Time PCR system (Applied Biosystems), in the Lightcycler 480 (Roche), or in the Biorad_CFX384 Real-Time detection system (Biorad). The primer sequences used are listed in Table 7. The data were normalized to expression of the cellular housekeeping genes, *GAPDH* or actin *(ACT)*.

Table 7. Primers used in this study.

Primer against	sequence/reference	source	used in
ACT	TCACCCACACTGTGCCATCTACGA, CAGCGGAACCGCTCATTGCCAATGG	Oligomer	III, IV
CD31	AACAGTGTTGACATGAAGAGCC, TGTAAAACAGCACGTCATCCTT	Oligomer	IV
CD34	TGGGCATCACTGGCTATTTC, CCACGTGTTGTCTTGCTGAA	Oligomer	IV
CDH2	Hs00169953_m1	Applied Biosystems	III
CDK6	CCAGATGGCTCTAACCTCAGT, AACTTCCACGAAAAAGAGGCTT	Oligomer	I
CNN1	Hs00154543_m1	Applied Biosystems	III
COL1A1	Hs00164004_m1	Applied Biosystems	III
CXCL12	TGCCAGAGCCAACGTCAA, CAGCCGGCCGGCTAC	Oligomer	IV
CXCR4	Hs00976734_m1	Applied Biosystems	III
	GCCAACGTCAGTGAGGCAGA, GCCAACCATGATGTGCTGAAAC	Oligomer	IV
DLL4	CTGGAGCTCAGCGAGTGTGAC, CCTGGTCCTTACAGCTGCCTC	Oligomer	IV
ETS2	Hs00232009_m1	Applied Biosystems	III
FLT4	Hs01047679_m1	Applied Biosystems	III
	GACAGCTACAAATACGAGCATCTG, CTGTCTTGCAGTCGAGCAGAA	Oligomer	IV
FOXF1	Hs00230962_m1	Applied Biosystems	III
FOXF2	Hs00230963_m1	Applied Biosystems	III
Gapdh	Mm03302249_g1	Applied Biosystems	I
	TCAACGACCCCTTCATTGAC, ATGCAGGGATGATGTTCTGG	Oligomer	I
GAPDH	Hs03929097_m1	Applied Biosystems	I, III, IV
	TCACCACCATGGAGAAGGCT, GCCATCCACAGTCTTCTGGG	Oligomer	I, IV
GFP	AAGCTGACCCTGAAGTTCATCTGC, CTTGTAGTTGCCGTCGTCCTTGAA	Oligomer	IV
Hes1	Mm01342805_m1	Applied Biosystems	I
HES1	Hs00172878_m1	Applied Biosystems	I, III
	TCAACACGACACCGGATAAA, TCAGCTGGCTCAGACTTTCA	Oligomer	IV
Hey1	Mm00468865_m1	Applied Biosystems	I
HEY1	Hs00232618_m1	Applied Biosystems	I, III
	GTTCGGCTCTAGGTTCCATGT, CGTCGGCGCTTCTCAATTATTC	Oligomer	IV
HEY2	TTGAGAAGACTTGTGCCAACTG, GTGCGTCAAAGTAGCCTTTACC	Oligomer	IV
JAG1	TGCCAAGTGCCAGGAAGT, GCCCCATCTGGTATCACACT	Oligomer	IV
K8.1	AAAGCGTCCAGGCCACCACAGA, TTCACTGCTGGTATCTGGAACG	Oligomer	III
LANA	CGGAGCTAAAGAGTCTGGTG, GCAGTCTCCAGAGTCTTCTC	Oligomer	III
MMP14	Hs01037009_m1	Applied Biosystems	III

	GCAGAAGTTTTACGGCTTGCAA, CCTTCGAACATTGGCCTTGAT	Oligomer	IV
MMP15	Hs00233997_m1	Applied Biosystems	III
MMP16	Hs00234676_m1	Applied Biosystems	III
***Notch*1**	CCGTGTAAGAATGCTGGAACG, AGCGACAGATGTATGAAGACTCA	Oligomer	I
NOTCH1	GAGGCGTGGCAGACTATCATGC, CTTGTACTCCGTCAGCGTGA	Oligomer	I, IV
NOTCH2	CCTGGGCTATACTGGGAGCTACTG, ACACCCTGATAGCCTGGGACAC	Oligomer	IV
Notch3	Mm00435270_m1	Applied Biosystems	I
NOTCH3	QT00003374	Qiagen	I, IV
NOTCH4	AATCCCACTGCCTCCAGACT, TTGTGGCAAAGGGAAGAGAC	Oligomer	IV
NRARP	Hs01104102_m1	Applied Biosystems	III
ORF25	GTCCACCCCTTCTTTGATTTTT, TTTCCCGAGTTGACCCAGTAGG	Oligomer	III
ORF50	CACAAAAATGGCGCAAGATGA, TGGTAGAGTTGGGCCTTCAGTT	Oligomer	III
PAI1	CAGGAAGCCCCTAGAGAACC, ATGCGGGCTGAGACTATG AC	Oligomer	III
PDGFRA	Hs00183486_m1	Applied Biosystems	III
PDGFRB	Hs01019589_m1	Applied Biosystems	III
PROX1	Hs00896294_m1	Applied Biosystems	III
	TGTTCACCAGCACACCCGCC, TCCTTCCTGCATTGCACTTCCCG	Oligomer	IV
pSIN	GTTTGCTACTGGAAAAAGAGGAAAGAG, AGGGCTGCTACGCTGCC	Oligomer	III
S100A4	Hs00243202_m1	Applied Biosystems	III
SNAI1	AATCGGAAGCCTAACTACAGCG, GTCCCAGATGAGCATTGGCA	Oligomer	III
SNAI2	Hs00950344_m1	Applied Biosystems	III
SPP1	Hs00959010_m1	Applied Biosystems	III
TAGLN	Hs00162558_m1	Applied Biosystems	III
v-cyclin	CGGACGTCACTTCCTTCTTG, CGCAGATCAAAGTCCGAAAC	Oligomer	I
VEGFC	GCAGTTACGGTCTGTGTCCA, TGTAATTGGTGGGGCAGGTC	Oligomer	IV
vFLIP	GCGGGCACAATGAGTTATTT, GGCGATAGTGTTGGGAGTGT	Oligomer	III
vGPCR	TGGCCCAAACGGAGGATCCTAG, AGTTTCATTCCAGGATTCATCATC	Oligomer	III
vIL-6	AAAACACGCACCGCTTGACCTG, TTCACTGCTGGTATCTGGAACG	Oligomer	III
VIM	Hs00958816_m1	Applied Biosystems	III
vIRF-2	CGGAATGGCTCACGGACTTTAT, AGACATCCTTCACATCCCTTGT	Oligomer	III

ZEB1	GATGATGAATGCGAGTCAGATGC, ACAGCAGTGTCTTGTTGTTGTAG	Oligomer	III

12. Global gene expression analyses (III, IV)

Total RNA was extracted using the RNeasy mini kit (Qiagen) or with Trizol isolation protocol (Sigma) supplemented with acid phenol-chloroform precipitation step. The RNA integrity was analyzed by Bioanalyzer (Agilent). In III, Affymetrix Hg-U133 plus 2.0 microarrays were used according to manufacturer's procedures, and the genome annotations were taken from the Bioconductor's repository package 'hgu133plus2.db'. In IV, the RNA sequencing was done by using the standard protocols and the Illumina NextSeq500 sequencer, the reads were aligned to HS GRCh38.76 reference genome, and Bioconductor's DESeq2 package was used to determine the differentially expressed genes from the count data. In both analyses, p-values less than 0.05 were considered significant.

13. Statistical Analysis (I, II, III, IV)

For statistical analysis of the qRT-PCR data logarithmic values were converted to ddCt values (log2 scale values) and p-values were calculated with a one-tailed unpaired Student's t-test. The p-values for FACS data were calculated directly from the data normalized to the appropriate control.

RESULTS AND DISCUSSION

1. KSHV V-CYCLIN EXPRESSION LEADS TO DIFFERENTIATION DEFECTS IN LYMPHOCYTE COMPARTMENT *IN VIVO* (I,II)

1.1 v-cyclin expression under the Eμ-promoter/enhancer leads to T-cell lymphoma dependent on Cdk6

To study viral cyclin mediated oncogenesis mechanisms *in vivo* (I, II), a previously described model in which v-cyclin expression was targeted to lymphocytes using the Eμ-promoter/enhancer in a mixed CBA/C57BL6 background was utilized. This resulted in the development of B- and T-cell lymphomas in 17% of the mice, which was accelerated by breeding the mice with the p53 null background (Verschuren et al., 2004a, Verschuren et al., 2002). However, since wild type p53 is usually retained in KSHV associated lymphomas (Sarek et al., 2007), better models for studying the v-cyclin induced lymphomagenesis were needed. Our results showed that the disease progression and survival of the mice was dependent on the mouse background, as breeding these mice into an ICR (CD1) outbred stock and the inbred C57BL6 strain had different consequences. In the ICR strain, v-cyclin expression led to poor survival, as less than 5% of the mice were alive after a follow-up period of 300 days, even though p53 and p19ARF were found not to be mutated in the mouse background (Fig. 1A in I). Backcrossing the mice into the C57BL6 strain resulted in reversion of the survival rate to similar as found in the original mixed background (Fig. S1A in I). This might be reflecting the intrinsic resistance to tumor formation described earlier in the C57BL6 mouse strain (Meuwissen and Berns, 2005), or the two-fold higher expression levels of v-cyclin seen in the ICR background (Fig. S1B in I).

The low survival rates of the ICR-v-cyclin mice were due to lymphomas mainly in the thymus and spleen, and pancarditis manifested as lymphocyte masses in the heart causing destruction of the heart muscle and failure of the hemodynamics (Fig. 1B and 1F in I). The lymphomas formed in the ICR-v-cyclin mice were of T-cell origin, although the expression of v-cyclin was hundreds of folds higher in the B-cells than T-cells (Fig. 1C in I). Since v-cyclin is known to induce DNA damage and apoptosis in cell culture models when overexpressed (Koopal et al., 2007, Ojala et al., 1999, Ojala et al., 2000, Verschuren et al., 2002), it is possible that tight regulation of the v-cyclin levels is needed for the tumor formation. Another explanation is that the pathways v-cyclin is affecting are important for the T-cell but not B-cell development, and thus their deregulation would lead primarily to formation of a T-cell type malignancy. The most well-characterized cellular partner of v-cyclin, CDK6, is known to be important for T-cell differentiation, and to be a driver of the T-cell lymphomagenesis (Chilosi et al., 1998, Grossel and Hinds, 2006b, Grossel and Hinds, 2006a, Hu et al., 2011, Sawai et al., 2012). Our results support the role of Cdk6 on participating in the v-cyclin induced T-cell lymphomas. Cdk6 was the predominant kinase in phosphorylating the tested *in vitro* substrates, GST-Rb and histone H1, together with v-cyclin when the freshly isolated mouse splenocytes were analyzed (Fig. 2D in I). Moreover, v-cyclin induced lymphomas were sensitive for the CDK4/6

kinase inhibitor, PD0332991, since the inhibitor treatment led to a cell cycle arrest and induced cell death in the lymphoma cell lines examined (Fig. 3A-B in I). To test whether the active CDK6-(v)-cyclin complexes were needed for the cell survival in the context of whole virus infection, the PD0332991 inhibitor was tested on patient derived, naturally KSHV infected PEL cells. CDK6 inhibition led to significant cell death in the BCBL-1 PEL cell line (Fig. 3D in I). These results thus suggest that the use of CDK4/6 inhibitors could be beneficial in KSHV associated malignancies and perhaps in cancers where the D type cyclin or interphase CDK (CDK4/6) expression is upregulated. In recent years, interphase CDKs have proven to be promising drug targets, as they are not frequently mutated in cancer but most cancer cells need their function, whereas they are not essential for the survival of normal cells (Musgrove et al., 2011). CDK4/6 kinase inhibitors have proven relatively safe in clinical trials, and are tested as therapeutics in many cancer types, including estrogen receptor (ER)-positive breast cancer and chronic lymphocytic leukemia (Brower, 2014). Hence, their trial in the KSHV associated malignancies, and especially PEL, would be justifiable as well.

1.2 T-cell development is distorted by v-cyclin expression

When the v-cyclin induced lymphomas were further analyzed by FACS and antibodies against CD4/CD8 T-cell subpopulations, most of the lymphomas exhibited a CD4-CD8 double negative phenotype (Fig. 1D in I). This suggests that a v-cyclin induced oncogenic event took place at the early phase of the T-lymphocyte maturation and might additionally hinder the T-lymphocyte development, given that differentiation and tumorigenesis are closely linked processes (Aifantis et al., 2008). To detect possible defects in the maturation of the lymphocytes, the thymi and spleens of the Eμ-v-cyclin mice were analyzed before the onset of the lymphomas. The size and cellularity of these lymphoid organs were significantly diminished when compared to the littermate control animals (Fig. 4A-B and S4A-B in I), indicating that v-cyclin expression was affecting the survival of some or all of the lymphocyte subtypes. When the thymocytes and splenocytes were subjected to analyses on proliferation, apoptosis, and cell cycle by FACS, the results indicated that the turnover of the v-cyclin expressing cells had increased (Fig. 4C-E in I). This implies that the decreased lymphoid organ size did not result from increased apoptosis or decrease in proliferation. Supporting the role of v-cyclin in disturbing T-cell differentiation, the v-cyclin expressing animals showed diminished CD3 expression over B220 expression when the spleens were analyzed by IHC and FACS (Fig. S4C-D), indicating that the T-lymphocytes did not reach the secondary lymphoid organs. When the T-cell maturation in the thymus was analyzed by FACS, the number of CD4-CD8 double negative and double positive, as well CD8 single positive cells had decreased. Peculiarly, the number of CD4 single positive cells had increased (Fig. 5C in I), and these cells were mostly pan-T-cell marker CD3 negative (Fig. 5F in I). When compared to thymocytes of the littermates, both the thymocytes and lymphoma cell lines isolated from the Eμ-v-cyclin mice showed a ten-fold increased expression of the plasma cell marker CD138 (Fig. 1E and 5G in I), which is also expressed by the PEL cells (Carbone et al., 2000, Gaidano et al., 1997). Taken together, v-cyclin expression leads not only to a block in the differentiation of the T-lymphocytes but also induces cell populations normally not

present in the thymi. As PEL cells exhibit an intermediate plasma cell phenotype (Klein et al., 2003), and PELs expressing T-cell markers have been characterized (Goto et al., 2013, Nepka et al., 2012, Said et al., 1999), it is possible that v-cyclin accounts for some of the differentiation alterations seen in this KSHV induced malignancy. In general, this is another example of how an oncogene expression can lead to differentiation defects, which then further leads to tumorigenesis.

1.3 v-cyclin induces the proinflammatory NF-κB pathway via Cdk6 dependent phosphorylation

Since about half of the lethally diseased Eμ-v-cyclin mice exhibited signs of pancarditis, we hypothesized that v-cyclin could affect pathways related to inflammation. The NF-κB pathway has been heavily linked to (auto)immunity and cancer (Hoesel and Schmid, 2013, Sun et al., 2013), as well as to KSHV pathogenesis (de Oliveira et al., 2010). As all the currently known v-cyclin functions are mediated by CDK6 dependent phosphorylations, we wanted to decipher whether the v-cyclin-CDK6 complex could phosphorylate the NF-κB effectors. By using an unbiased approach based on ion exchange chromatography after IL-1 stimulation, it had been previously shown that the NF-κB effector p65 could be phosphorylated at S536 (pS536-p65) by five distinct kinases (Buss et al., 2004). These kinases included IKKα, IKKβ, IKKε, TRAF family member-associated-binding kinase 1 (TBK1), and an unknown kinase (Buss et al., 2004). In our study, further fractionation and mass spectrometry analysis revealed that the identity of the unknown kinase was CDK6 (Fig. 1 in II). Furthermore, pS536-p65 co-immunoprecipitated with CDK6, and *in vitro* kinase assay showed that recombinant CDK6 together with v-cyclin could phosphorylate the same site on p65 (Fig. 2 in II). Inhibition of CDK6 by silencing or chemical inhibitors in cell models led to attenuation in TNF/IL-1 stimulated p65 S536 phosphorylation, a decrease in NF-κB luciferase reporter signal, and a decrease in the expression of NF-κB target genes (Fig. 3A-C and 4 in II). Moreover, CDK6 silencing in PEL cells led to downregulation of p65 phosphorylation at S536 in the nucleus (Fig. 3D-E in II). Hence, v-cyclin-CDK6 complex in virus context is activating and fine-tuning the NF-κB pathway, and thus most probably regulates the inflammatory response seen in KS/PEL. This is particularly intriguing given that the PEL cells have been shown to be dependent on constitutive NF-κB activity, vFLIP has been shown to induce the pathway via IKK (Guasparri et al., 2004, Keller et al., 2000), and there has been reports showing that other KSHV encoded proteins can modify the NF-κB pathway as well (de Oliveira et al., 2010). Taken together, these results reveal that KSHV regulates the NF-κB pathway by several different mechanisms. This highlights the importance of the pathway not only for KSHV immune evasion but also for the KSHV mediated induction of malignancies through NF-κB pro-tumorigenic effects.

In vivo, pS536-p65 was induced in the v-cyclin expressing pre-tumorigenic lymphatic organs of the Eμ-v-cyclin mice and even more pronouncedly in the thymic lymphomas when compared to the littermate controls (Fig. 6A-B in II). As this correlated with the CDK6 levels of the given organs, and since CDK6 gave the highest kinase activity with v-cyclin in an *in vitro* kinase assay performed using the isolated Eμ-v-cyclin splenocytes

(Fig. 2D in I), it is probable that the phosphorylation of p65 on S536 is mediated by CDK6. CDK6 can, however, phosphorylate p65 and other targets also with the cellular cyclin D, and cyclin D3 was significantly induced in the v-cyclin expressing lymphoid organs and lymphomas (Fig. 6A in II and Fig. 6E in I). Thus, even though the *in vitro* data suggests that v-cyclin-CDK6 complex is inducing p65 phosphorylation slightly more potently than CDK6-cyclin D complexes (Fig. 2A in II), it cannot be ruled out that part of the pS536-p65 seen in the lymphoid organs and thymic lymphomas of the Eμ-v-cyclin mice would be the consequence of increased activation of the CDK6-cyclin D complex. In either case, as the isolated lymphoma cell lines from the Eμ-v-cyclin mice treated with CDK4/6 kinase inhibitors showed compromised survival, it is tempting to postulate that part of the survival mechanism in the Eμ-v-cyclin lymphomas is mediated by the CDK6 dependent NF-κB activation. Moreover, if the increased phosphorylation of p65 leads to similar cytokine response *in vivo* as in the cell culture models, it is plausible to surmise that activated NF-κB could mediate the lethal pancarditis seen in the Eμ-v-cyclin mice.

1.4 Notch pathway activation accounts for T-cell defects *in vivo*

The Notch pathway has been shown to be one of the most important regulators of T-cell fate (Lefort et al., 2006), and *NOTCH1* is the most often mutated gene in the human T-cell acute lymphoblastic leukemia T-ALL (Weng et al., 2004). As it has been shown that Notch signaling supports the survival of the KSHV lymphoma cells (Lan et al., 2009), we hypothesized that the Notch signaling pathway might be involved in causing the observed defects in the T-cell development as well as T-cell lymphoma seen in the Eμ-cyclin mice. To this end, the expression levels of Notch receptors and their targets from the pre-tumorigenic tissues and lymphomas isolated from the Eμ-cyclin mice were analyzed. From the Notch receptors, Notch1 and Notch3 were expressed in the mouse thymocytes. Notch3 and its intracellular activated domain NICD3 were upregulated both in the v-cyclin expressing thymocytes and thymic lymphomas when compared to the littermate controls, whereas Notch1 expression was solely upregulated in the lymphoma phase (Fig. 6A-C in I). Appropriately, the Notch downstream targets *Hes1* and *Hey1* were upregulated already in the pre-tumorigenic phase, and *Hes1* expression had further increased in the thymic lymphomas (Fig. 6D in I), whereas *Hey2* and *Hes5* were not expressed at the mouse thymocytes. These results suggest that the increased amounts of activated Notch3 could cause the defects in the T-cell development and participate in the initiation of the lymphomas, whereas Notch1 upregulation seems to be secondary to the lymphomagenesis but could still further increase the downstream target expression and be crucial for the lymphoma survival. Further corroborating evidence on the functional significance of the activated Notch signaling in the Eμ-cyclin lymphomas came from the assays using the gamma secretase inhibitor DAPT, which prevents the cleavage of the Notch receptor to an active intracellular domain and leads to abortion of the Notch target gene expression. Treating the isolated Eμ-cyclin lymphoma cells with DAPT led to cell cycle arrest and increased cell death, suggesting that constitutively active Notch signaling was required for the survival of the Eμ-cyclin lymphoma cells (Fig. 7C in I). Earlier studies have shown that PEL cells in culture and in xenograft models are dependent on activated Notch (Lan et al., 2009) and suggested that treating KSHV associated malignancies with gamma

secretase inhibitors might be beneficial. Our results further show that v-cyclin can participate in the Notch activation in an appropriate context and, thus, support the use of these inhibitors to treat these malignancies.

To study if the v-cyclin induced Notch activation was dependent on CDK6, its expression was silenced in a human cell culture model. As v-cyclin expression in cell culture can lead to DNA damage, cellular senescence, or apoptosis, we chose to study the role of CDK6 in a HEK293 cell line, which has NOTCH3 expression already without v-cyclin. We silenced CDK6 expression by lentivirus-mediated shRNA, which led to downregulation of *NOTCH3* and *HES1* in this model (Fig. 6F in I), implying that the Notch activation can indeed be dependent on CDK6. In conclusion, the results show that cyclin-CDK can have a role in the initiation of Notch-dependent lymphomagenesis, possibly through interference with the development of the lymphocytes. It has been previously shown that Notch increases cell survival through induction of cyclin D3 expression in T-cell malignancies (Joshi et al., 2009, Sicinska et al., 2003, Choi et al., 2012). In the Eμ-cyclin mice, the cyclin D3 expression is induced, and we show that a cyclin can act as an upstream regulator of Notch. These findings argue against cyclins being solely downstream effectors of cell proliferation and raise a question whether cellular cyclins can also function upstream of Notch and possibly other pathways. Indeed, there has been a report showing that cyclin D1 can act as an upstream transcriptional regulator of Notch1 in a developing mouse eye (Bienvenu et al., 2010). Nevertheless, the mechanism leading to Notch activation seems to be different from our model, since the Notch1 expression in mouse retina did not require CDK6 function, but cyclin D1 expression was found to directly lead to RBP-Jκ binding at the Notch1 promoter (Bienvenu et al., 2010).

2. TRANSDIFFERENTIATION OF PRIMARY LYMPHATIC ENDOTHELIAL CELLS CONTRIBUTES TO CELLULAR HETEROGENEITY IN KAPOSI'S SARCOMA (III)

2.1 Kaposi's sarcoma exhibits cellular heterogeneity

It has been known that Kaposi's sarcoma (KS) tumors express a variety of different cellular lineage markers, including markers of blood and lymphatic endothelial cells, fibroblasts, smooth muscle cells, and several immune system cells (Kaaya et al., 1995, Sturzl et al., 1995, Weich et al., 1991). As the tumors contain multiple cell types, we wanted to test whether the cells expressing the mesenchymal/fibroblast markers were KSHV infected tumor cells. We double stained human KS tumor sections for KSHV latent antigen LANA and several of the mesenchymal lineage markers. The majority of the KS spindle cells were found to co-express LANA and vimentin as well as LANA and PDGFR-α, while a subgroup of the spindle cells simultaneously expressed LANA and α-SMA (Fig. 1, S1 and Table S1 in III). These results demonstrated that the KS tumor cells were heterogeneous in their lineage marker expression, and even though they are thought to be of endothelial origin and express several endothelial lineage markers, they also express markers of smooth muscle cells and fibroblasts. The possible explanations for the

heterogeneous lineage marker expression are that 1) several different cell types can be infected and transformed, 2) a common progenitor cell is transformed, or 3) KSHV causes transdifferentiation of the originally infected cell. It does not seem likely that a simultaneous transformation process would occur in multiple cell types and finally result in similar morphology of the tumor cells. In turn, it has been shown that especially human mesenchymal stem cells are not easily infected *in vitro*, suggesting that the progenitor cells might not be the major target cells of KSHV (unpublished observation by E. Kaivanto and ref. (Yoo et al., 2014)). Thus, the most probable explanation for the different lineage marker expression is that endothelial cells can transdifferentiate towards mesenchymal fate or vice versa. As EndMT is a morphogenic process used in development, the endothelial cells are infected with KSHV rather easily, and the prevailing view of the KSHV origin supports endothelial cells (Cancian et al., 2013), we hypothesized that EndMT was contributing to the cellular heterogeneity seen in the KS tumors.

2.2 3D culture of KSHV infected LECs leads to reprogramming towards mesenchymal cell fate

Upon KSHV infection, endothelial cells adopt spindle morphology and exhibit a vast array of gene expression changes, reviewed in (Cancian et al., 2013). In BECs, it has been shown that KSHV infection can lead to expression of LEC markers, whereas KSHV infected LECs (K-LEC) acquire BEC markers (Carroll et al., 2004, Hong et al., 2004, Wang et al., 2004). This suggests that KSHV infected endothelial cells are able to undergo cellular transdifferentiation. However, fibroblast/smooth muscle cell marker expression in spindle cells was not explained by these findings. To study if this was due to cell culture conditions not permissive for transdifferentiation beyond the endothelial cell fate, we cultured K-LECs in 3D conditions, better mimicking the cellular milieu *in vivo*. As KS tumors contain a myriad of leaky vessels and thus grow in environment rich in fibrin, we chose to use cross-linked fibrin matrix and adapted an assay developed for angiogenesis studies (Korff and Augustin, 1998), where preformed EC clusters called spheroids are embedded into fibrin matrix. Interestingly, embedding the K-LEC spheroids into the fibrin matrix resulted in frequent outgrowth of the cells (sprouting) from the spheroid body, whereas uninfected LEC spheroids did not sprout (Fig. 2A in III). These sprouts did not represent lymphangiogenesis as they had lost expression of endothelial cell markers, but instead gained expression of fibroblast and smooth muscle cell markers such as α-SMA, vimentin, N-cadherin, transgelin, and fibronectin (Fig. 2B-C and S2). Furthermore, known EMT/EndMT effectors Snail and PDGFR-β were upregulated in the K-LEC spheroids (Fig. 2C and S3B in III). Thus, it seemed that a portion of the K-LECs in contact with the 3D matrix had undergone EndMT. This finding explains the high cellular heterogeneity seen in KS tumors and supports the role of LECs as a source of KS spindle cells. As mesenchymal cells are generally more motile than endothelial cells, EndMT might be a frequent event needed for the spread of the endothelial cell derived tumor cells in KS. In other cancer types where endothelial cells are not directly affected by cancer initiating mutations, its role is more obscure. However, reports from Kalluri and colleagues have described that EndMT can serve as a major source of CAFs in melanoma and pancreatic

cancer (Zeisberg et al., 2007a), and thus facilitate the tumor progression also in other contexts beyond KS.

2.3 Angiogenesis and EndMT are opposing events which are balanced in the tumors

In addition to the peculiar KS spindle cells, the heterogeneity of the KS tumors involves angiogenesis and leaky blood vessels, both phenotypes resulting from increased VEGF signaling (Koch et al., 2011). Furthermore, it has been shown that VEGF is secreted by the KS tumor cells (Cornali et al., 1996, Masood et al., 1997, Nakamura et al., 1997), and that KSHV lytic gene vGPCR expression can lead to increased expression of VEGF and its receptor VEGFR-2 (Jham and Montaner, 2010, Bais et al., 2003, Sodhi et al., 2004a, Sodhi et al., 2000). Additionally, other factors, including Angiopoietin-like 4 (ANGPTL4), have also been shown to participate in the increased angiogenesis and vascular permeability induced by KSHV vGPCR (Ma et al., 2010). To study the effect of high concentration of angiogenic factors on the transdifferentiation phenotype, the 3D K-LEC cultures were stimulated with VEGF or VEGF-C. Adding either of the growth factors led to increased sprouting from the spheroid body compared to the non-stimulated K-LEC spheroid. However, these sprouts expressed markers of (lymph)angiogenesis, namely PECAM, and did not express α-SMA or fibronectin, which were in turn expressed by the non-stimulated spheroid sprouts (Fig. 3G in III). Thus, the endothelial growth factors at high levels can override the EndMT phenotype and lead to increased angiogenic sprouting. This fits to the KS context where both phenotypes are present, probably representing the different ends of the VEGF gradients in the microenvironment, or intrinsic differences in the susceptibility of the tumor cells to the angiogenic factors, bearing in mind that the VEGF receptors and other endothelial markers can also be downregulated by KSHV (Carroll et al., 2004, Hong et al., 2004, Wang et al., 2004, Mansouri et al., 2006, Qian et al., 2008) and (Fig. S2 in III). Furthermore, hypoxic conditions seen in the KS tumor microenvironment can further complex the cell fate determination. Hypoxia has been shown to lead to increased angiogenesis through HIF-1α-VEGF axis (Baeriswyl and Christofori, 2009), but it can also serve as an initiator of EMT (Higgins et al., 2007, Jiang et al., 2011) and is a physiological stimulus of KSHV reactivation (Davis et al., 2001). Reactivation can lead to increased viral loads and expression of hundreds of viral genes, which can in turn affect the EndMT process as well as angiogenesis. Indeed, we detected that inside the spheroid body, which represents the more hypoxic environment, there was an increase in the expression of viral lytic genes (Fig. 7B-C). Generation of new virus particles could possibly facilitate the spread of the virus and balance the switch between angiogenesis and EndMT by currently unknown mechanisms.

2.4 Notch pathway activity is required for EndMT by KSHV

Mechanisms leading to EndMT in the cancer context are not well understood, but it is known that the major pathways driving EndMT in development are TGF-β and Notch pathways (Garside et al., 2013). We tested the role of these pathways in KSHV induced EndMT by using different chemical inhibitors. The Notch pathway inhibition by DAPT or inhibitor of soluble Dll4/Notch signaling (Dll4-Fc) led to decreased sprouting of the K-LEC spheroids and downregulated expression of mesenchymal markers in qRT-PCR (Fig. 3E-F and S3D-E in III). On the other hand, an inhibitor of the TGF-β downstream kinases (SB431542) or a TGF-β blocking antibody did not have any effect on KSHV-induced EndMT (Fig. 3E and S3A-C in III). These results indicated that in our model system Notch, not TGF-β, pathway activation is necessary for the EndMT induction. However, contribution of additional signaling pathways in conjunction with Notch cannot be excluded for the EndMT initiation or execution, as the endothelial cell culture media used in these studies is rich in growth factors such as FGF and was further supplemented with human serum. As inhibition of the Notch pathway has been linked to increased lymphangiogenesis (Zheng et al., 2011), Notch pathway together with VEGF levels might be the responsible for distinction between angiogenesis and EndMT in the KS context.

Notch signaling is overly active in the KSHV associated malignancies (KSHV induction of Notch signaling summarized in Figure 4): PEL cells have been shown to be dependent on activated Notch signaling (Lan et al., 2009), whereas Notch receptors (Notch1-4) and targets have been reported to be upregulated in the KS tumors (Curry et al., 2007, Curry et al., 2005). In LECs, it has been further shown that KSHV vFLIP and vGPCR can activate Notch signaling by inducing the expression of the pathway ligands Jag1 and Dll4 through NF-κB and ERK signaling, respectively (Emuss et al., 2009). In our system, lentiviral expression of either vFLIP or vGPCR in LEC led to a mesenchymal phenotype reminiscent of the EndMT caused by KSHV in a Notch dependent manner (Fig. 4A-D in III). Silencing of vFLIP or vGPCR by siRNA from the K-LEC spheroids again led to decreased mesenchymal sprouting (Fig. 4F-G in III). To study whether the Notch pathway was upstream of the known EndMT effector PDGFR-β that was upregulated by the KSHV infection, we analyzed its expression levels after Notch inhibition, and it was found to be downregulated (Fig. 3F and S3E in III). Furthermore, its silencing led to inhibited EndMT without affecting the Notch downstream targets (Fig. 6D-E in III), thus suggesting that it indeed functions downstream of Notch. These findings suggest that it is indeed the viral genes that are driving the EndMT process through Notch (summarized in figure 7) and leading to the activation of the EndMT executor PDGFR-β.

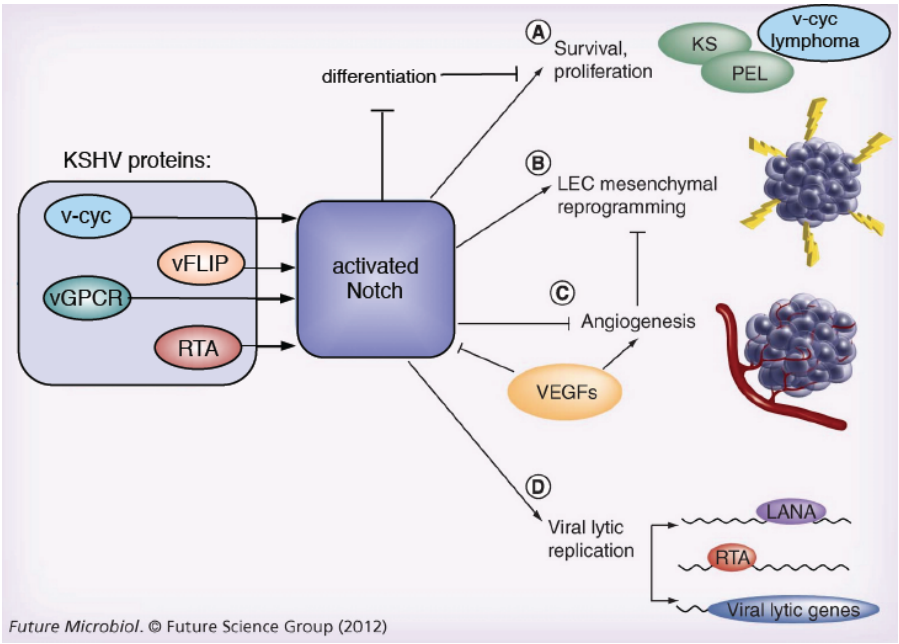

Figure 7. Notch functions in KS/PEL pathogenesis.

KSHV proteins vGPCR, vFLIP, RTA, and v-cyclin (v-cyc) activate Notch signaling. This leads to increased survival and proliferation of the tumor cells (A), and, at least in some contexts, this is due to inhibited differentiation. In addition, Notch activation leads to EndMT in infected LECs (B) and inhibits angiogenesis (C). Furthermore, Notch activation increases KSHV lytic replication (D) (modified from (Cheng et al., 2012)).

2.5 Increased MMP activity by KSHV in the tumors enables invasion and spread of the infected cells

To further analyze the effect of the KSHV expression in 3D LECs, we did a global gene expression analysis of the 3D cultured K-LECs and the parental non-infected LECs. The microarray analysis showed, that invasion related genes such as *SPP1*, *ETS2*, *CXCR4,* and *PDGFRA* were upregulated in K-LEC spheroids (Fig. 5A-B in III). Furthermore, the K-LEC cells invaded into 3D collagen ten-fold more effectively than the control LECs (Fig. 5D in III). These results suggested that the EndMT process in 3D increased the invasive properties of the K-LECs, and this might be important for the spread of the infected cells, thus allowing further expansion of the infection and the tumor progression. Indeed, it has been later shown that KSHV can regulate several analogical invasion/motility related molecules, including CXCR7 and ETS-1 (Gutierrez et al., 2013, Raggo et al., 2005, Totonchy et al., 2014). As MMPs are key enzymes in cancer invasion (Egeblad and Werb, 2002), and MMP-1, -2, and -9 had been shown to be upregulated in the KSHV infected HUVECs (Qian et al., 2007), we deciphered their role in the EndMT related invasion by using specific inhibitors. Global MMP inhibitors, GM6001 and TIMP2, resulted in a

decrease of the K-LEC spheroid sprouting, whereas TIMP1, an inhibitor of the secreted MMPs, did not (Fig. 5C in III). This implied that one of the three membrane-type (MT) MMPs would be causing the increased invasiveness. We could detect that KS tumors co-expressed LANA and MT1-MMP in the tumor mass, as well as in the single cells invading into the surrounding stroma (Fig. 5E in III). K-LEC spheroids overexpressed MT1-MMP when compared to the LEC control, and siRNA silencing of MT1-MMP in the spheroid system led to decreased sprouting and reduced expression of *PDGFRB* and *TAGLN* (Fig. 5F and 6A in III). MT1-MMP silencing did not significantly affect the Notch targets, whereas Notch inhibition led to decreased MT1-MMP expression (Fig. 6B-C in III), suggesting that MT1-MMP is acting downstream of Notch. In conclusion, our findings revealed the K-LEC invasion in 3D matrix requires initiation of EndMT by activated Notch signaling, followed by activation of EndMT effectors such as PDGFR-β, and invasion related enzymes including MT1-MMP (summarized in Figure 8). Later, other studies have additionally shown that KSHV can indeed promote EndMT through Notch and transcription factors Slug and ZEB1 activation (Gasperini et al., 2012).

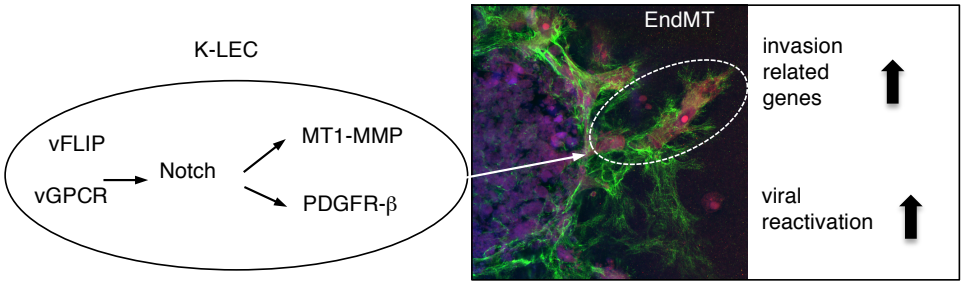

Figure 8. KSHV induced EndMT.

KSHV vFLIP and vGPCR expression in LECs activates Notch signaling, which further leads to EndMT via MT1-MMP and PDGFR-β upregulation. In addition, EndMT in K-LECs leads to upregulation of invasion related genes and increased viral reactivation, thus potentially facilitating the viral spread. A K-LEC spheroid stained with antibodies against fibronectin (green) and podoplanin (red) is shown. Nuclei were counterstained with Hoechst (blue).

3. MELANOMA METASTASIS IS AUGMENTED BY LEC – MELANOMA CELL INTERACTION (IV)

3.1 3D LEC-melanoma co-culture system

Tumor microenvironment has become increasingly recognized to be an important regulator of tumor progression (Quail and Joyce, 2013). In melanoma, the presence of lymph node metastasis is considered as one of the main prognostic factors (Balch et al., 2009), but the removal of the metastatic lymph nodes does not lead to survival benefit (Morton et al., 2006). This suggests that the drivers of the tumor progression occur before the melanoma cells have reached the lymph nodes, or that the contact of the tumor cells with the stromal cells within the lymph nodes leads to further changes in cancer progression that are not abolished by removing the lymph nodes. As the primary melanomas and lymph node metastases are abundant in lymphatic vessels, and the amount of lymphatic vessels and lymphatic vessel invasion in the primary tumors and lymph nodes negatively correlate with the prognosis (Dadras et al., 2005, Dadras et al., 2003), we hypothesized that LEC-melanoma cell interaction could lead to changes in the melanoma progression. This could occur through LEC induced changes in melanoma, melanoma induced changes in LECs, or both. To this end, we developed a method to study the melanoma interaction with primary LECs in a cross-linked 3D matrix. The preformed LEC spheroids were embedded into fibrin matrix together with GFP expressing melanomas as single cells (Fig. 1A in IV). In this assay, melanoma cell lines with high or low metastatic capacity reacted differentially. Bowes melanoma cells isolated from the superficially spreading melanoma increased the LEC sprouting (Fig. 1B in IV), which was likewise evident when the LECs were stimulated with their growth factor, VEGF-C (data not shown). Thus, the melanoma cells with low initial metastatic capacity increased the lymphangiogenic potential of the LECs. The melanoma cells from a metastatic skin lesion of nodular melanoma (WM852), on the other hand, inhibited the spontaneous or VEGF-C induced LEC sprouting, suggesting that the lymphangiogenesis is no longer needed at the metastatic phase of the melanoma (Fig. 1B in IV). Importantly, both of the cell lines were able to make space for themselves in the LEC spheroid body by downregulating endothelial cell markers such as endothelial adhesion molecule PECAM on LECs (Fig. 1C in IV). The capability of the melanoma cells to invade into the LEC spheroids seemed to be independent of the intrinsic metastatic properties of the cells. The seemingly different effects of the melanoma cells on the spheroids could reflect the different origins of these cell lines. The cell line from the early stage melanoma could still need the increased lymphangiogenesis to maximize its contact with the lymphatic vasculature and thereby facilitate its spread, whereas the already metastatic cell line only needs the invasive capacities.

3.2 Melanoma interaction leads to loss of LEC cell-cell contact and identity markers

As both of the melanoma cell lines were able to invade into the LEC spheroid, the lymphatic invasion capacity seemed to be a general feature in melanoma. To study what are the processes needed for this invasion, we first assessed if the LEC-melanoma

interaction would alter the LECs to allow the melanoma invasion. The EC adherent junction molecule, VE-cadherin, was downregulated in LECs in contact with the melanoma cells both in 3D and 2D (Fig. 2A-B in IV). This suggests that the melanoma cells reduced integrity in the LEC spheroids/monolayer. The phenotype was dependent on cell-cell contacts, as the conditioned media from LEC or LEC-melanoma co-culture did not lead to altered VE-cadherin expression (Fig. S2 in IV). As the melanoma cells express a plethora of proteases (Moro et al., 2014), it was not surprising that the cell surface markers of LECs were altered in the presence of melanoma. In fact, it has already earlier been shown that cancer cells are able to downregulate endothelial markers, if the cancer cell spheroids are cultivated on top of a monolayer of endothelial cells (Kerjaschki et al., 2011). However, in addition to VE-cadherin, we could detect that Prox-1, the master regulator of the lymphatic endothelial cell fate, was downregulated in the LECs that were in contact with the melanoma cells (Fig. 2C in IV). This suggests that the melanoma cells are able to alter the cell fate of the LECs in a manner that could contribute to the cancer cell invasion.

3.3 LEC interaction gives rise to invasive properties in melanoma cells

To decipher whether the LEC-melanoma interaction in the co-culture would give rise to changes in the invasive properties, the cell migration and adhesive capabilities of the cells were studied. The 2D cell migration was studied by using a modified wound healing assay, where melanoma cells and LECs were seeded onto the opposite sides of the wound. In the Bowes-LEC assay, this resulted in accelerated wound closure compared to the wound healing assays containing only either one of the cell types on both sides (Fig. S1C). On the contrary, WM852 wound closure was not affected by the presence of the LECs (data not shown). As it seemed that the faster wound closure of the Bowes-LEC assay was due to increased LEC migration, and WM852-LEC did not show any phenotype, it is well probable that this is due to the increased lymphangiogenic potential of the Bowes co-cultured LECs seen in 3D rather than increased cancer cell migration. The adhesion of the melanoma cells or LECs onto a LEC layer before and after co-culture were studied by seeding single-cultured LECs or melanoma cells, or co-cultured cells on top of a confluent LEC layer as depicted in Fig. 3A in IV. The single-cultured LECs formed small colonies on top of the LEC layer and showed signs of apoptosis, whereas most of the single-cultured melanoma cells that adhered disrupted the bottom LEC layer, attached to the bottom of the plate, and displayed flattened morphology. Strikingly, the LEC co-cultured melanoma cells rather attached to each other and formed large sphere like structures that were partially surrounded by the LECs, while the cell attachment to the plate was abolished (Fig. 3B in IV). The phenotype was more pronounced in the metastatic WM852 cell line, which suggests that the co-culture of melanoma with LEC had changed their adhesive properties to each other especially when the tumor cell background was aggressive. Surprisingly, seeding of the co-cultured melanoma cells additionally led to downregulation of VE-cadherin, PECAM, and Prox-1 in the bottom LEC layer (Fig. 3C in IV), suggesting that changes in the LEC fate and identity might be contributing to the increased adhesion of the tumor cells.

To specifically assess the invasive capabilities of the co-cultured melanoma cells, we decided to use the more aggressive WM852 cell line showing more pronounced phenotype in the adhesion assay, and separated them from LECs after the co-culture by using magnetic nanoparticles (see workflow, Fig. 4A in IV). After the separation, the LEC co-cultured (LEC primed) melanoma cells were subjected to adhesion assay as well as fibrin invasion assay. The adhesion assay revealed that the melanoma cells had gained capabilities during the co-culture, which allowed them to adhere together, attract cells from the underlying LEC monolayer, as well as downregulate the endothelial markers in the LEC monolayer (Fig. 4B in IV). Furthermore, after embedding the single cells into fibrin, the single-cultured WM852 remained as round single cells, whereas the LEC primed WM852 cells started exhibiting elongated morphology already at 24 hours. While the WM852 cells cultured alone started forming small, round cell clusters over several days in fibrin, the LEC primed WM852 cells retained the elongated morphology, formed structures where cells lined up after each other, and showed increased invasive potential (Fig. 4C-D in IV). Taken together, these findings suggest that the adhesive and invasive capabilities of the melanoma cells were enhanced by the LEC co-culture, highlighting the impact of tumor-stroma interactions in gaining tumor progression traits.

To decipher the molecular mechanisms behind the increased invasive potential of the co-cultured melanoma cells, we analyzed some of the genes known to be involved in the melanoma invasion and metastasis. qRT-PCR analysis of the LEC primed WM852 cells revealed that the expression levels of CXCR4 and MT1-MMP were upregulated when normalized to single-cultured WM852 cells (Fig. 5A and S4A in IV). We were able to show that LEC conditioned medium was sufficient to upregulate CXCR4 both at mRNA and protein level in the WM852 cells (Fig. 5B-C in IV), suggesting that a secreted factor would explain this difference. Indeed, CXCR4 ligand CXCL12 was found to be upregulated in the WM852 primed LECs, and inhibition of CXCR4 ligand binding partially reverted the LEC co-culture mediated adhesion properties of the melanoma cells (Fig. 5D-E in IV). Fittingly, it has been earlier shown that LECs secreting the CXCL12 in metastatic sites recruit CXCR4+ melanoma cells, thus augmenting the melanoma metastasis *in vivo* (Kim et al., 2010). MT1-MMP expression was not altered in the WM852 cells treated with the LEC conditioned media, implying that the physical co-culture gives rise to additional changes in the expression of invasion related genes possibly reflecting the different outcome of conditioned media and contact mediated changes for the invasion related phenotypes.

When we analyzed the global gene expression of the LEC primed WM852 by RNA sequencing, we observed that a large portion of genes was differentially expressed from the WM852 cells cultured alone (Fig. 6A in IV). These genes included members of the Notch pathway (Fig. 6B in IV), which is known to induce changes in melanoma progression through activation of cell proliferation pathways and promoting adhesive and invasive properties, as well as to increase cell plasticity and metastasis (Balint et al., 2005, Liu et al., 2006, Bedogni et al., 2008, Asnaghi et al., 2012, Howard et al., 2013, Hardy et al., 2010, Topczewska et al., 2006). The qRT-PCR validation showed that LECs induce Notch pathway activation in melanoma through *NOTCH3* receptor and target genes *HES1*,

HEY1, and *HEY2* (Fig. 6C in IV). Moreover, inhibition of the Notch pathway by DAPT led to reversion of the co-culture mediated adhesion phenotype (Fig. 6E in IV). As a less pronounced effect was observed with the inhibitor of CXCR4 ligand binding, it is tempting to speculate that the Notch pathway could elicit part of its functions in melanoma through CXCR4-CXCL12 axis, similar to multiple myeloma (Mirandola et al., 2013). The function of Notch in melanoma development/progression is summarized in Figure 9.

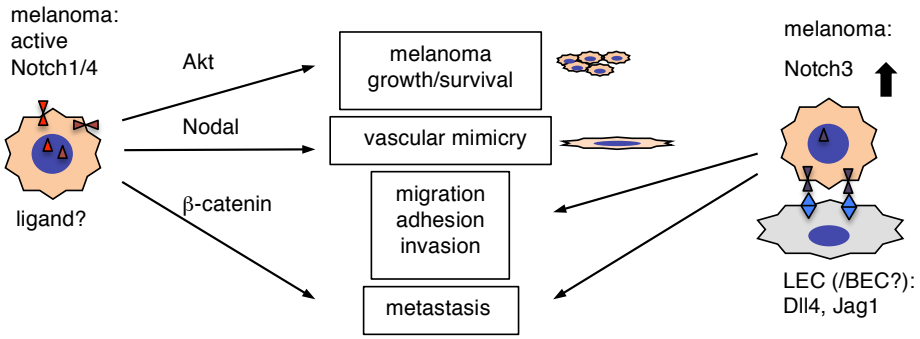

Figure 9. Notch regulation in melanoma.

Notch1 or Notch4 activation has been shown to lead to activation of Akt, Nodal, and β-catenin in melanoma, which increases melanoma cell survival and growth, vascular mimicry, and metastasis. We show that Notch3 in melanoma can be upregulated by the LEC contact, possibly through the elevated Dll4/Jag1 ligand expression on LEC. Melanoma-LEC contact leads to activation of adhesion, invasion, and metastasis.

3.4 LEC-melanoma interaction leads to a more metastatic phenotype *in vivo*

To study the tumorigenic and metastatic capacity of melanoma cells after LEC priming *in vivo*, we injected the GFP-luciferase expressing 3D single-cultured or 3D LEC primed melanoma cells subcutaneously into immunocompromized Scid mice (see workflow in Fig. 7A in IV). The primary tumor growth rates did not change upon LEC priming, and the size of the WM852 tumors at the time of collection/sacrifice were similar with or without LEC priming, whereas the 3D LEC primed Bowes tumors were slightly larger at the end point (Fig. S6B-C in IV). IHC analysis of the tumors revealed that 3D LEC priming facilitated the stromal cell recruitment to the primary tumors, as the 3D LEC priming led to increased recruitment of α-SMA and fibronectin positive fibroblasts to the WM852 and Bowes tumors (Fig. 7C in IV). As these markers represent activated fibroblasts, these cells could represent the cancer associated fibroblasts (CAFs), known mediators of tumor progression (Augsten, 2014). Thus, in addition to the direct LEC mediated effects on melanoma cells affecting the tumor cell adhesion and invasion, the LEC priming led to indirect effects on the tumor progression through changes in the tumor microenvironment.

Furthermore, when the metastases were analyzed by postmortem luciferase imaging, the LEC priming was found to significantly increase distant organ metastasis of the LEC primed WM852 tumors, whereas the LEC primed or non-primed Bowes cell tumors showed no distant organ metastasis (Fig. 7F-G and S6E in IV). These results suggest that LEC priming of the melanoma cells leads to alterations in the tumor stroma and increased metastatic capacity of the aggressive and plastic tumor cells *in vivo*. In mice with the Bowes cell tumors, distant organ metastasis was not initiated by the LEC priming. Thus, it is possible that the stromal cells can only unleash the already existing metastatic capacity of the tumor cells. Similarly, it has been shown that breast cancer cells can condition lymphatics in the pre-metastatic sites to generate a pre-metastatic niche and promote metastasis (Lee et al., 2014a, Lee et al., 2014b). In summary, the lymphatics - tumor cell interactions might be a general mechanism contributing to the metastasis cascade in collaboration with the acquired mutations of the tumor cells.

CONCLUSIONS AND FUTURE PERSPECTIVES

This thesis has implicated that tumor cell plasticity and cellular reprogramming can contribute to several steps in tumor progression. Tumor initiation can be triggered if the differentiation of the pre-tumorigenic cell lineage is hindered while the proliferation pathways are active. Moreover, false timing of morphogenetic processes such as EMT and EndMT can induce increased tumor invasion as well as give rise to tumor microenvironmental cells like CAFs. In addition, the metastasis cascade can be activated by tumor-stroma interactions, which reveal the plastic and aggressive nature of the tumor cells. The Notch pathway activation can participate in all these processes and regulate the malignant cascade by affecting the tumor cell plasticity. This further implies that certain signaling pathways can regulate multiple steps of tumorigenesis.

In future, it is critical to reveal what the key mechanisms involved in regulating tumor cell plasticity are, so that the spread of the cancers and the resulting morbidity and mortality could be diminished. Additionally, it is important to understand how these mechanisms are regulated, and how the tumor-stroma interactions affect the tumorigenesis to further understand the full complexity of the malignant progression. As this study revealed that the Notch pathway can be induced by both virus-mediated mechanisms as well as through tumor-stroma interaction, it further corroborates the idea that at least KSHV and possibly other human tumor viruses cause cancers as side effects of their life cycle regulation by affecting pathways needed in both processes. Moreover, these results and reports of others show how viruses have evolved to deregulate the signaling pathways important for their spread by several, partially overlapping and complementary mechanisms. Hence, the tumor viruses continue to be extremely helpful in understanding the tumorigenesis process in general. They contain a restricted set of genes, which have an essential function for the viral life cycle and spread, and thus possibly for the malignant progression as well. Accordingly, by studying which pathways they regulate in a redundant fashion might reveal the important pathways critical for driving the tumor progression out of the wide range of changes in the tumor cells.

ACKNOWLEDGEMENTS

This thesis was carried out in 2007-2015 in the Genome-Scale Biology and Translational Cancer Biology Research Programs, Research Programs Unit, Faculty of Medicine, University of Helsinki, as well as at the Institute of Biotechnology, University of Helsinki. I would like to thank the former and current leaders of these departments for providing inspirational and well-equipped environment for conducting my studies.

The Doctoral Programme In Biomedicine (DPBM) in Doctoral School in Health Sciences (DSHealth) is acknowledged for their financial support, and especially for organizing the M.D. Ph.D. program. It allowed me to be part of the scientific community already from the first year of my M.D. studies, and continued to support me after my graduation from the Medical School in 2010. In addition, the following organizations gave me financial support to finish my Ph.D.: The Finnish Medical Foundation, The Finnish Cancer Foundation, and Emil Aaltonen Foundation.

I am grateful for my supervisor Päivi Ojala for giving me the opportunity to join a vivid community in her lab, and allowing me to grow and become a researcher during these years. I admire her for being so passionate and enthusiastic about the results, and I am thankful for her that she spread her excitement to me as well. I have not only learned a vast amount of techniques but also a new way to think.

I would like to thank the reviewers of my thesis, docent Eeva Auvinen and Professor Sirpa Jalkanen, for providing insightful comments. I acknowledge Professor Enrique Mesri for accepting the invitation to be my Opponent and Professor Tomi Mäkelä for being the Custos. Tiia Pelkonen is acknowledged for the well-conducted linguistic review. My thesis committee members Professor Pirjo Laakkonen and docent Petteri Arstila are thanked for their support especially at the early phases of my work. Kaisa Sarkkinen and Satu Sankkila, thank you for excellently arranged practicalities.

I acknowledge all the collaborators and co-authors in Finland and abroad; your help has been crucial. Professors Kari Alitalo, Lauri Aaltonen, Sampsa Hautaniemi, Peter Biberfeld, Adam Grundhoff, Chris Boshoff, Michael Kracht, Jukka Westermarck, Gerard Evan, and Ethel Cesarman are granted for providing reagents and support. Specially, I would like to thank docent Kaisa Lehti, who has been involved in two of my projects and helped me tremendously, as well as docent Emmy Verschuren, whose help was needed to finalize the Eμ-v-cyclin mouse project. In addition, I would like to acknowledge Ville Rantanen, Nami Sugiyama, Stephen Henderson, and Mervi Aavikko for being part of the EndMT project; Anna Cvrljevic and Sonam Prakash for being involved in the Eμ-v-cyclin mouse project; and Olga Tatti for time and help in the melanoma project.

Being part of the Ojala lab, I have had the pleasure to encounter many people who have influenced my thinking on both academic and non-academic topics. Annika, I am very happy that it was you who I had an opportunity to work with when I started my Ph.D., but I am even more delighted that we have kept in contact and stayed as friends ever since. Rose and Simonas, we were a good team. Simonas, special thanks for introducing the dark room to me. Nadya, I admire your precision, and I am grateful for your help in everything. Elisa, I wish we would have had better luck, but it was still a good experience to work with you. Johanna V, we shared the path for a long time. I am glad that we could support each other when things were not easy, but also that we had fun in the lab and outside, abroad and home. Jenni, my forest company, it has been nice to have you around. Nina, I liked working with you, I think it really worked well, and hope that it

would have lasted longer. However, I am happy that you found your path. Giuseppe, thank you for infecting me with optimism, it has helped me a lot. Markus and Anne L, thanks for your good ideas. Jenny and Sari, thank you for being the sane ones and for all the excellent assistance. Thank you Sonja, Grzegorz, Mary (Li), Raquel, Monica, Anne A, Liisa, Mari, Jaakko, and Leevi for fun company and help. Marine, Wale, Otso, and Krista, it has been a pleasure to supervise you, I hope I did not scare you badly ;).

I would also like to thank people from other labs, both in Biomedicum and Biocenter, who have helped me and created a nice atmosphere where to work at. Specifically, I would like to acknowledge Alex, Terhi, Karita, Irene, Saana, Kaisa, and Mikko for the corridor chats and/or fun meeting company.

My dear friends and family, I could not be more fortunate to have you around. My parents, Marja and Reijo, and little brother Pekka, thank you for supporting me in all possible ways. Mimmi, my grandmother, you have always been an example for me, and despite the 60-years in between we are alike. Jari, it feels good to be loved and cared for. Inkku, it has been more than half of our lives, unbelievable! It really means a lot to me to have you in my life, I could not think not being friends. Lauri T, thank you for your long lasting friendship, not everybody understands us, but who cares. Johanna H, I am happy that I have had a sane friend from a long time back, maybe Latin helped in something... Pia, art and trips rule. And a little gossip, or more. Lauri A, I am glad that we have stayed friends; your support was and is appreciated. Jaana, Anna V, Laura, Riitta, and Camilla, thank you for your friendship from high school and Medical School. Tuomas, Teemu, Mikko H, Olli, Jussi, Jasu, and Jukka, I am glad that I have met you and we have had a chance to spend fun time together. A special thanks to my ballet friends, Tuuli, Anna L, Anita, Linda, Mari, Suvi, Lotta, Johanna A, Helena, Rauni, Mirja, Krista, Ida, Noora, Taru, and all the rest, we have spent quite some time together and experienced a lot. Lastly, I would like to thank my dear cat babies, Hilla and Nestori.

Helsinki 2015

Pirita Pekkonen

BIBLIOGRAPHY

AAVIKKO, M. 2014. Identification of novel tumor predisposition families and underlying genetic defects. *Unigrafia, thesis*, ISBN: 978-951-51-0252-2.

ADAMS, J. M. 2003. Ways of dying: multiple pathways to apoptosis. *Genes Dev,* 17**,** 2481-95.

ADAMS, J. M. & CORY, S. 2007. The Bcl-2 apoptotic switch in cancer development and therapy. *Oncogene,* 26**,** 1324-37.

ADAMS, J. M., HARRIS, A. W., PINKERT, C. A., CORCORAN, L. M., ALEXANDER, W. S., CORY, S., PALMITER, R. D. & BRINSTER, R. L. 1985. The c-myc oncogene driven by immunoglobulin enhancers induces lymphoid malignancy in transgenic mice. *Nature,* 318**,** 533-8.

AEBISCHER, D., IOLYEVA, M. & HALIN, C. 2014. The inflammatory response of lymphatic endothelium. *Angiogenesis,* 17**,** 383-93.

AGUILAR, B., CHOI, I., CHOI, D., CHUNG, H. K., LEE, S., YOO, J., LEE, Y. S., MAENG, Y. S., LEE, H. N., PARK, E., KIM, K. E., KIM, N. Y., BAIK, J. M., JUNG, J. U., KOH, C. J. & HONG, Y. K. 2012. Lymphatic reprogramming by Kaposi sarcoma herpes virus promotes the oncogenic activity of the virus-encoded G-protein-coupled receptor. *Cancer Res,* 72**,** 5833-42.

AGUILAR, V. & FAJAS, L. 2010. Cycling through metabolism. *EMBO Mol Med,* 2**,** 338-48.

AIFANTIS, I., RAETZ, E. & BUONAMICI, S. 2008. Molecular pathogenesis of T-cell leukaemia and lymphoma. *Nat Rev Immunol,* 8**,** 380-90.

ALBRECHT, I. & CHRISTOFORI, G. 2011. Molecular mechanisms of lymphangiogenesis in development and cancer. *Int J Dev Biol,* 55**,** 483-94.

ALITALO, A. & DETMAR, M. 2012. Interaction of tumor cells and lymphatic vessels in cancer progression. *Oncogene,* 31**,** 4499-508.

ALITALO, K. 2011. The lymphatic vasculature in disease. *Nat Med,* 17**,** 1371-80.

ALKHARSAH, K. R., SINGH, V. V., BOSCO, R., SANTAG, S., GRUNDHOFF, A., KONRAD, A., STURZL, M., WIRTH, D., DITTRICH-BREIHOLZ, O., KRACHT, M. & SCHULZ, T. F. 2011. Deletion of Kaposi's sarcoma-associated herpesvirus FLICE inhibitory protein, vFLIP, from the viral genome compromises the activation of STAT1-responsive cellular genes and spindle cell formation in endothelial cells. *J Virol,* 85**,** 10375-88.

AN, J., SUN, Y., SUN, R. & RETTIG, M. B. 2003. Kaposi's sarcoma-associated herpesvirus encoded vFLIP induces cellular IL-6 expression: the role of the NF-kappaB and JNK/AP1 pathways. *Oncogene,* 22**,** 3371-85.

ARMSTRONG, E. J. & BISCHOFF, J. 2004. Heart valve development: endothelial cell signaling and differentiation. *Circ Res,* 95**,** 459-70.

ARVANITAKIS, L., GERAS-RAAKA, E., VARMA, A., GERSHENGORN, M. C. & CESARMAN, E. 1997. Human herpesvirus KSHV encodes a constitutively active G-protein-coupled receptor linked to cell proliferation. *Nature,* 385**,** 347-50.

ARVANITAKIS, L., MESRI, E. A., NADOR, R. G., SAID, J. W., ASCH, A. S., KNOWLES, D. M. & CESARMAN, E. 1996. Establishment and characterization of a primary effusion (body cavity-based) lymphoma cell line (BC-3) harboring kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8) in the absence of Epstein-Barr virus. *Blood,* 88**,** 2648-54.

ASHLOCK, B. M., MA, Q., ISSAC, B. & MESRI, E. A. 2014. Productively infected murine Kaposi's sarcoma-like tumors define new animal models for studying and targeting KSHV oncogenesis and replication. *PLoS One,* 9**,** e87324.

ASNAGHI, L., EBRAHIMI, K. B., SCHRECK, K. C., BAR, E. E., COONFIELD, M. L., BELL, W. R., HANDA, J., MERBS, S. L., HARBOUR, J. W. & EBERHART, C. G. 2012. Notch signaling promotes growth and invasion in uveal melanoma. *Clin Cancer Res,* 18**,** 654-65.

ASTER, J. C., XU, L., KARNELL, F. G., PATRIUB, V., PUI, J. C. & PEAR, W. S. 2000. Essential roles for ankyrin repeat and transactivation domains in induction of T-cell leukemia by notch1. *Mol Cell Biol,* 20**,** 7505-15.

AUGSTEN, M. 2014. Cancer-associated fibroblasts as another polarized cell type of the tumor microenvironment. *Front Oncol,* 4**,** 62.

AUGSTEN, M., HAGGLOF, C., OLSSON, E., STOLZ, C., TSAGOZIS, P., LEVCHENKO, T., FREDERICK, M. J., BORG, A., MICKE, P., EGEVAD, L. & OSTMAN, A. 2009. CXCL14 is an autocrine growth factor for fibroblasts and acts as a multi-modal stimulator of prostate tumor growth. *Proc Natl Acad Sci U S A,* 106**,** 3414-9.

AZHAR, M., RUNYAN, R. B., GARD, C., SANFORD, L. P., MILLER, M. L., ANDRINGA, A., PAWLOWSKI, S., RAJAN, S. & DOETSCHMAN, T. 2009. Ligand-specific function of transforming growth factor beta in epithelial-mesenchymal transition in heart development. *Dev Dyn,* 238**,** 431-42.

AZIMI, F., SCOLYER, R. A., RUMCHEVA, P., MONCRIEFF, M., MURALI, R., MCCARTHY, S. W., SAW, R. P. & THOMPSON, J. F. 2012. Tumor-infiltrating lymphocyte grade is an independent predictor of sentinel lymph node status and survival in patients with cutaneous melanoma. *J Clin Oncol,* 30**,** 2678-83.

BAERISWYL, V. & CHRISTOFORI, G. 2009. The angiogenic switch in carcinogenesis. *Semin Cancer Biol,* 19**,** 329-37.

BAIS, C., SANTOMASSO, B., COSO, O., ARVANITAKIS, L., RAAKA, E. G., GUTKIND, J. S., ASCH, A. S., CESARMAN, E., GERSHENGORN, M. C. & MESRI, E. A. 1998. G-protein-coupled receptor of Kaposi's sarcoma-associated herpesvirus is a viral oncogene and angiogenesis activator. *Nature,* 391**,** 86-9.

BAIS, C., VAN GEELEN, A., EROLES, P., MUTLU, A., CHIOZZINI, C., DIAS, S., SILVERSTEIN, R. L., RAFII, S. & MESRI, E. A. 2003. Kaposi's sarcoma associated herpesvirus G protein-coupled receptor immortalizes human endothelial cells by activation of the VEGF receptor-2/ KDR. *Cancer Cell,* 3**,** 131-43.

BALCH, C. M., GERSHENWALD, J. E., SOONG, S. J., THOMPSON, J. F., ATKINS, M. B., BYRD, D. R., BUZAID, A. C., COCHRAN, A. J., COIT, D. G., DING, S., EGGERMONT, A. M., FLAHERTY, K. T., GIMOTTY, P. A., KIRKWOOD, J. M., MCMASTERS, K. M., MIHM, M. C., JR., MORTON, D. L., ROSS, M. I., SOBER, A. J. & SONDAK, V. K. 2009. Final version of 2009 AJCC melanoma staging and classification. *J Clin Oncol,* 27**,** 6199-206.

BALINT, K., XIAO, M., PINNIX, C. C., SOMA, A., VERES, I., JUHASZ, I., BROWN, E. J., CAPOBIANCO, A. J., HERLYN, M. & LIU, Z. J. 2005. Activation of Notch1 signaling is required for beta-catenin-mediated human primary melanoma progression. *J Clin Invest,* 115**,** 3166-76.

BALLON, G., CHEN, K., PEREZ, R., TAM, W. & CESARMAN, E. 2011. Kaposi sarcoma herpesvirus (KSHV) vFLIP oncoprotein induces B cell transdifferentiation and tumorigenesis in mice. *J Clin Invest,* 121**,** 1141-53.

BECKER, K. F., ROSIVATZ, E., BLECHSCHMIDT, K., KREMMER, E., SARBIA, M. & HOFLER, H. 2007. Analysis of the E-cadherin repressor Snail in primary human cancers. *Cells Tissues Organs,* 185**,** 204-12.

BEDOGNI, B., WARNEKE, J. A., NICKOLOFF, B. J., GIACCIA, A. J. & POWELL, M. B. 2008. Notch1 is an effector of Akt and hypoxia in melanoma development. *J Clin Invest,* 118**,** 3660-70.

BENEDITO, R. & HELLSTROM, M. 2013. Notch as a hub for signaling in angiogenesis. *Exp Cell Res,* 319**,** 1281-8.

BENJAMIN, L. E., HEMO, I. & KESHET, E. 1998. A plasticity window for blood vessel remodelling is defined by pericyte coverage of the preformed endothelial network and is regulated by PDGF-B and VEGF. *Development,* 125**,** 1591-8.

BIENVENU, F., JIRAWATNOTAI, S., ELIAS, J. E., MEYER, C. A., MIZERACKA, K., MARSON, A., FRAMPTON, G. M., COLE, M. F., ODOM, D. T., ODAJIMA, J., GENG, Y., ZAGOZDZON, A., JECROIS, M., YOUNG, R. A., LIU, X. S., CEPKO, C. L., GYGI, S. P. & SICINSKI, P. 2010. Transcriptional role of cyclin D1 in development revealed by a genetic-proteomic screen. *Nature,* 463**,** 374-8.

BOCHET, L., LEHUEDE, C., DAUVILLIER, S., WANG, Y. Y., DIRAT, B., LAURENT, V., DRAY, C., GUIET, R., MARIDONNEAU-PARINI, I., LE GONIDEC, S., COUDERC, B., ESCOURROU, G., VALET, P. & MULLER, C. 2013. Adipocyte-derived fibroblasts promote tumor progression and contribute to the desmoplastic reaction in breast cancer. *Cancer Res,* 73**,** 5657-68.

BOHUSLAV, J., CHEN, L. F., KWON, H., MU, Y. & GREENE, W. C. 2004. p53 induces NF-kappaB activation by an IkappaB kinase-independent mechanism involving phosphorylation of p65 by ribosomal S6 kinase 1. *J Biol Chem,* 279**,** 26115-25.

BONDE, A. K., TISCHLER, V., KUMAR, S., SOLTERMANN, A. & SCHWENDENER, R. A. 2012. Intratumoral macrophages contribute to epithelial-mesenchymal transition in solid tumors. *BMC Cancer,* 12**,** 35.

BORGGREFE, T. & OSWALD, F. 2009. The Notch signaling pathway: transcriptional regulation at Notch target genes. *Cell Mol Life Sci,* 66**,** 1631-46.

BOSHOFF, C. & WEISS, R. 2002. AIDS-related malignancies. *Nat Rev Cancer,* 2**,** 373-82.

BOTTERO, V., SHARMA-WALIA, N., KERUR, N., PAUL, A. G., SADAGOPAN, S., CANNON, M. & CHANDRAN, B. 2009. Kaposi sarcoma-associated herpes virus (KSHV) G protein-coupled receptor (vGPCR) activates the ORF50 lytic switch promoter: a potential positive feedback loop for sustained ORF50 gene expression. *Virology,* 392**,** 34-51.

BOULANGER, E., GERARD, L., GABARRE, J., MOLINA, J. M., RAPP, C., ABINO, J. F., CADRANEL, J., CHEVRET, S. & OKSENHENDLER, E. 2005. Prognostic factors and outcome of human herpesvirus 8-associated primary effusion lymphoma in patients with AIDS. *J Clin Oncol,* 23**,** 4372-80.

BRADY, C. A. & ATTARDI, L. D. 2010. p53 at a glance. *J Cell Sci,* 123**,** 2527-32.

BRAEUER, R. R., ZIGLER, M., VILLARES, G. J., DOBROFF, A. S. & BAR-ELI, M. 2011. Transcriptional control of melanoma metastasis: the importance of the tumor microenvironment. *Semin Cancer Biol,* 21**,** 83-8.

BREITENEDER-GELEFF, S., SOLEIMAN, A., KOWALSKI, H., HORVAT, R., AMANN, G., KRIEHUBER, E., DIEM, K., WENINGER, W., TSCHACHLER, E., ALITALO, K. & KERJASCHKI, D. 1999. Angiosarcomas express mixed endothelial phenotypes of blood and lymphatic capillaries: podoplanin as a specific marker for lymphatic endothelium. *Am J Pathol,* 154**,** 385-94.

BROWER, V. 2014. Cell cycle inhibitors make progress. *J Natl Cancer Inst,* 106.

BUSS, H., DORRIE, A., SCHMITZ, M. L., HOFFMANN, E., RESCH, K. & KRACHT, M. 2004. Constitutive and interleukin-1-inducible phosphorylation of p65 NF-{kappa}B at serine 536 is mediated by multiple protein kinases including I{kappa}B kinase (IKK)-{alpha}, IKK{beta}, IKK{epsilon}, TRAF family member-associated (TANK)-binding kinase 1 (TBK1), and an unknown kinase and couples p65 to TATA-binding protein-associated factor II31-mediated interleukin-8 transcription. *J Biol Chem,* 279**,** 55633-43.

CABIOGLU, N., YAZICI, M. S., ARUN, B., BROGLIO, K. R., HORTOBAGYI, G. N., PRICE, J. E. & SAHIN, A. 2005. CCR7 and CXCR4 as novel biomarkers predicting axillary lymph node metastasis in T1 breast cancer. *Clin Cancer Res,* 11**,** 5686-93.

CAMPOS-SANCHEZ, E. & COBALEDA, C. 2014. Tumoral reprogramming: Plasticity takes a walk on the wild side. *Biochim Biophys Acta*.

CANCIAN, L., HANSEN, A. & BOSHOFF, C. 2013. Cellular origin of Kaposi's sarcoma and Kaposi's sarcoma-associated herpesvirus-induced cell reprogramming. *Trends Cell Biol,* 23**,** 421-32.

CARBONE, A., CILIA, A. M., GLOGHINI, A., CAPELLO, D., FASSONE, L., PERIN, T., ROSSI, D., CANZONIERI, V., DE PAOLI, P., VACCHER, E., TIRELLI, U., VOLPE, R. & GAIDANO, G. 2000. Characterization of a novel HHV-8-positive cell line reveals implications for the pathogenesis and cell cycle control of primary effusion lymphoma. *Leukemia,* 14**,** 1301-9.

CARMELIET, P. & JAIN, R. K. 2011. Molecular mechanisms and clinical applications of angiogenesis. *Nature,* 473**,** 298-307.

CARROLL, P. A., BRAZEAU, E. & LAGUNOFF, M. 2004. Kaposi's sarcoma-associated herpesvirus infection of blood endothelial cells induces lymphatic differentiation. *Virology,* 328**,** 7-18.

CESARMAN, E., CHANG, Y., MOORE, P. S., SAID, J. W. & KNOWLES, D. M. 1995a. Kaposi's sarcoma-associated herpesvirus-like DNA sequences in AIDS-related body-cavity-based lymphomas. *N Engl J Med,* 332**,** 1186-91.

CESARMAN, E., MOORE, P. S., RAO, P. H., INGHIRAMI, G., KNOWLES, D. M. & CHANG, Y. 1995b. In vitro establishment and characterization of two acquired immunodeficiency syndrome-related lymphoma cell lines (BC-1 and BC-2) containing Kaposi's sarcoma-associated herpesvirus-like (KSHV) DNA sequences. *Blood,* 86**,** 2708-14.

CESARMAN, E., NADOR, R. G., BAI, F., BOHENZKY, R. A., RUSSO, J. J., MOORE, P. S., CHANG, Y. & KNOWLES, D. M. 1996. Kaposi's sarcoma-associated herpesvirus contains G protein-coupled receptor and cyclin D homologs which are expressed in Kaposi's sarcoma and malignant lymphoma. *J Virol,* 70**,** 8218-23.

CHABOTTAUX, V., RICAUD, S., HOST, L., BLACHER, S., PAYE, A., THIRY, M., GAROFALAKIS, A., PESTOURIE, C., GOMBERT, K., BRUYERE, F., LEWANDOWSKY, D., TAVITIAN, B., FOIDART, J. M., DUCONGE, F. & NOEL, A. 2009. Membrane-type 4 matrix metalloproteinase (MT4-MMP) induces lung metastasis by alteration of primary breast tumour vascular architecture. *J Cell Mol Med,* 13**,** 4002-13.

CHANG, A. C., GARSIDE, V. C., FOURNIER, M., SMRZ, J., VRLJICAK, P., UMLANDT, P., FULLER, M., ROBERTSON, G., ZHAO, Y., TAM, A., JONES, S. J., MARRA, M. A., HOODLESS, P. A. & KARSAN, A. 2014. A Notch-dependent transcriptional hierarchy promotes mesenchymal transdifferentiation in the cardiac cushion. *Dev Dyn,* 243**,** 894-905.

CHANG, P. H., HWANG-VERSLUES, W. W., CHANG, Y. C., CHEN, C. C., HSIAO, M., JENG, Y. M., CHANG, K. J., LEE, E. Y., SHEW, J. Y. & LEE, W. H. 2012. Activation of Robo1 signaling of breast cancer cells by Slit2 from stromal fibroblast restrains tumorigenesis via blocking PI3K/Akt/beta-catenin pathway. *Cancer Res,* 72**,** 4652-61.

CHANG, Y., CESARMAN, E., PESSIN, M. S., LEE, F., CULPEPPER, J., KNOWLES, D. M. & MOORE, P. S. 1994. Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. *Science,* 266**,** 1865-9.

CHANG, Y., MOORE, P. S., TALBOT, S. J., BOSHOFF, C. H., ZARKOWSKA, T., GODDEN, K., PATERSON, H., WEISS, R. A. & MITTNACHT, S. 1996. Cyclin encoded by KS herpesvirus. *Nature,* 382**,** 410.

CHENG, F., PEKKONEN, P. & OJALA, P. M. 2012. Instigation of Notch signaling in the pathogenesis of Kaposi's sarcoma-associated herpesvirus and other human tumor viruses. *Future Microbiol,* 7**,** 1191-205.

CHI, J. T., CHANG, H. Y., HARALDSEN, G., JAHNSEN, F. L., TROYANSKAYA, O. G., CHANG, D. S., WANG, Z., ROCKSON, S. G., VAN DE RIJN, M., BOTSTEIN, D. & BROWN, P. O. 2003. Endothelial cell diversity revealed by global expression profiling. *Proc Natl Acad Sci U S A,* 100**,** 10623-8.

CHIANG, M. Y., XU, L., SHESTOVA, O., HISTEN, G., L'HEUREUX, S., ROMANY, C., CHILDS, M. E., GIMOTTY, P. A., ASTER, J. C. & PEAR, W. S. 2008. Leukemia-associated NOTCH1 alleles are weak tumor initiators but accelerate K-ras-initiated leukemia. *J Clin Invest,* 118**,** 3181-94.

CHILOSI, M., DOGLIONI, C., YAN, Z., LESTANI, M., MENESTRINA, F., SORIO, C., BENEDETTI, A., VINANTE, F., PIZZOLO, G. & INGHIRAMI, G. 1998. Differential expression of cyclin-dependent kinase 6 in cortical thymocytes and T-cell lymphoblastic lymphoma/leukemia. *Am J Pathol,* 152**,** 209-17.

CHIN, L. 2003. The genetics of malignant melanoma: lessons from mouse and man. *Nat Rev Cancer,* 3**,** 559-70.

CHOI, Y. J., LI, X., HYDBRING, P., SANDA, T., STEFANO, J., CHRISTIE, A. L., SIGNORETTI, S., LOOK, A. T., KUNG, A. L., VON BOEHMER, H. & SICINSKI, P. 2012. The requirement for cyclin D function in tumor maintenance. *Cancer Cell,* 22**,** 438-51.

CHUGH, P., MATTA, H., SCHAMUS, S., ZACHARIAH, S., KUMAR, A., RICHARDSON, J. A., SMITH, A. L. & CHAUDHARY, P. M. 2005. Constitutive NF-kappaB activation, normal Fas-induced apoptosis, and increased incidence of lymphoma in human herpes virus 8 K13 transgenic mice. *Proc Natl Acad Sci U S A,* 102**,** 12885-90.

COBALEDA, C., JOCHUM, W. & BUSSLINGER, M. 2007. Conversion of mature B cells into T cells by dedifferentiation to uncommitted progenitors. *Nature,* 449**,** 473-7.

COFFELT, S. B., HUGHES, R. & LEWIS, C. E. 2009. Tumor-associated macrophages: effectors of angiogenesis and tumor progression. *Biochim Biophys Acta,* 1796**,** 11-8.

CONANT, K. L., MARINELLI, A. & KALEEBA, J. A. 2013. Dangerous liaisons: molecular basis for a syndemic relationship between Kaposi's sarcoma and P. falciparum malaria. *Front Microbiol,* 4**,** 35.

CORNALI, E., ZIETZ, C., BENELLI, R., WENINGER, W., MASIELLO, L., BREIER, G., TSCHACHLER, E., ALBINI, A. & STURZL, M. 1996. Vascular endothelial growth factor regulates angiogenesis and vascular permeability in Kaposi's sarcoma. *Am J Pathol,* 149**,** 1851-69.

COURTOIS, G. & GILMORE, T. D. 2006. Mutations in the NF-kappaB signaling pathway: implications for human disease. *Oncogene,* 25**,** 6831-43.

CURRY, C. L., REED, L. L., BROUDE, E., GOLDE, T. E., MIELE, L. & FOREMAN, K. E. 2007. Notch inhibition in Kaposi's sarcoma tumor cells leads to mitotic catastrophe through nuclear factor-kappaB signaling. *Mol Cancer Ther,* 6**,** 1983-92.

CURRY, C. L., REED, L. L., GOLDE, T. E., MIELE, L., NICKOLOFF, B. J. & FOREMAN, K. E. 2005. Gamma secretase inhibitor blocks Notch activation and induces apoptosis in Kaposi's sarcoma tumor cells. *Oncogene,* 24**,** 6333-44.

CURTIN, J. A., FRIDLYAND, J., KAGESHITA, T., PATEL, H. N., BUSAM, K. J., KUTZNER, H., CHO, K. H., AIBA, S., BROCKER, E. B., LEBOIT, P. E., PINKEL, D. & BASTIAN, B. C. 2005. Distinct sets of genetic alterations in melanoma. *N Engl J Med,* 353**,** 2135-47.

DADRAS, S. S., LANGE-ASSCHENFELDT, B., VELASCO, P., NGUYEN, L., VORA, A., MUZIKANSKY, A., JAHNKE, K., HAUSCHILD, A., HIRAKAWA, S., MIHM, M. C. & DETMAR, M. 2005. Tumor lymphangiogenesis predicts melanoma metastasis to sentinel lymph nodes. *Mod Pathol,* 18**,** 1232-42.

DADRAS, S. S., PAUL, T., BERTONCINI, J., BROWN, L. F., MUZIKANSKY, A., JACKSON, D. G., ELLWANGER, U., GARBE, C., MIHM, M. C. & DETMAR, M. 2003. Tumor lymphangiogenesis: a novel prognostic indicator for cutaneous melanoma metastasis and survival. *Am J Pathol,* 162**,** 1951-60.

DAI, L., TRILLO-TINOCO, J., BAI, L., KANG, B., XU, Z., WEN, X., DEL VALLE, L. & QIN, Z. 2014. Systematic analysis of a xenograft mice model for KSHV+ primary effusion lymphoma (PEL). *PLoS One,* 9**,** e90349.

DAS, S., SARROU, E., PODGRABINSKA, S., CASSELLA, M., MUNGAMURI, S. K., FEIRT, N., GORDON, R., NAGI, C. S., WANG, Y., ENTENBERG, D., CONDEELIS, J. & SKOBE, M. 2013. Tumor cell entry into the lymph node is controlled by CCL1 chemokine expressed by lymph node lymphatic sinuses. *J Exp Med,* 210**,** 1509-28.

DAVIES, H., BIGNELL, G. R., COX, C., STEPHENS, P., EDKINS, S., CLEGG, S., TEAGUE, J., WOFFENDIN, H., GARNETT, M. J., BOTTOMLEY, W., DAVIS, N., DICKS, E., EWING, R., FLOYD, Y., GRAY, K., HALL, S., HAWES, R., HUGHES, J., KOSMIDOU, V., MENZIES, A., MOULD, C., PARKER, A., STEVENS, C., WATT, S., HOOPER, S., WILSON, R., JAYATILAKE, H., GUSTERSON, B. A., COOPER, C., SHIPLEY, J., HARGRAVE, D., PRITCHARD-JONES, K., MAITLAND, N., CHENEVIX-TRENCH, G., RIGGINS, G. J., BIGNER, D. D., PALMIERI, G., COSSU, A., FLANAGAN, A., NICHOLSON, A., HO, J. W., LEUNG, S. Y., YUEN, S. T., WEBER, B. L., SEIGLER, H. F., DARROW, T. L., PATERSON, H., MARAIS, R., MARSHALL, C. J., WOOSTER, R., STRATTON, M. R. & FUTREAL, P. A. 2002. Mutations of the BRAF gene in human cancer. *Nature,* 417**,** 949-54.

DAVIS, D. A., RINDERKNECHT, A. S., ZOETEWEIJ, J. P., AOKI, Y., READ-CONNOLE, E. L., TOSATO, G., BLAUVELT, A. & YARCHOAN, R. 2001. Hypoxia induces lytic replication of Kaposi sarcoma-associated herpesvirus. *Blood,* 97**,** 3244-50.

DE OLIVEIRA, D. E., BALLON, G. & CESARMAN, E. 2010. NF-kappaB signaling modulation by EBV and KSHV. *Trends Microbiol,* 18**,** 248-57.

DELHASE, M., KIM, S. Y., LEE, H., NAIKI-ITO, A., CHEN, Y., AHN, E. R., MURATA, K., KIM, S. J., LAUTSCH, N., KOBAYASHI, K. S., SHIRAI, T., KARIN, M. & NAKANISHI, M. 2012. TANK-binding kinase 1 (TBK1) controls cell survival through PAI-2/serpinB2 and transglutaminase 2. *Proc Natl Acad Sci U S A,* 109**,** E177-86.

DERYUGINA, E. I. & QUIGLEY, J. P. 2006. Matrix metalloproteinases and tumor metastasis. *Cancer Metastasis Rev,* 25**,** 9-34.

DING, X. Y., DING, J., WU, K., WEN, W., LIU, C., YAN, H. X., CHEN, C., WANG, S., TANG, H., GAO, C. K., GUO, L. N., CAO, D., LI, Z., FENG, G. S., WANG, H. Y. & XU, Z. F. 2012. Cross-talk between endothelial cells and tumor via delta-like ligand 4/Notch/PTEN signaling inhibits lung cancer growth. *Oncogene,* 31**,** 2899-906.

DISIS, M. L. 2010. Immune regulation of cancer. *J Clin Oncol,* 28**,** 4531-8.

DITTMER, D., LAGUNOFF, M., RENNE, R., STASKUS, K., HAASE, A. & GANEM, D. 1998. A cluster of latently expressed genes in Kaposi's sarcoma-associated herpesvirus. *J Virol,* 72**,** 8309-15.

DOMENGA, V., FARDOUX, P., LACOMBE, P., MONET, M., MACIAZEK, J., KREBS, L. T., KLONJKOWSKI, B., BERROU, E., MERICSKAY, M., LI, Z., TOURNIER-LASSERVE, E., GRIDLEY, T. & JOUTEL, A. 2004. Notch3 is required for arterial identity and maturation of vascular smooth muscle cells. *Genes Dev,* 18**,** 2730-5.

DULAUROY, S., DI CARLO, S. E., LANGA, F., EBERL, G. & PEDUTO, L. 2012. Lineage tracing and genetic ablation of ADAM12(+) perivascular cells identify a major source of profibrotic cells during acute tissue injury. *Nat Med,* 18**,** 1262-70.

DUPIN, N., FISHER, C., KELLAM, P., ARIAD, S., TULLIEZ, M., FRANCK, N., VAN MARCK, E., SALMON, D., GORIN, I., ESCANDE, J. P., WEISS, R. A., ALITALO, K. & BOSHOFF, C. 1999. Distribution of human herpesvirus-8 latently infected cells in Kaposi's sarcoma, multicentric Castleman's disease, and primary effusion lymphoma. *Proc Natl Acad Sci U S A,* 96**,** 4546-51.

DZIERZAK, E. & SPECK, N. A. 2008. Of lineage and legacy: the development of mammalian hematopoietic stem cells. *Nat Immunol,* 9**,** 129-36.

EGEBLAD, M. & WERB, Z. 2002. New functions for the matrix metalloproteinases in cancer progression. *Nat Rev Cancer,* 2**,** 161-74.

EGER, A., AIGNER, K., SONDEREGGER, S., DAMPIER, B., OEHLER, S., SCHREIBER, M., BERX, G., CANO, A., BEUG, H. & FOISNER, R. 2005. DeltaEF1 is a transcriptional repressor of E-cadherin and regulates epithelial plasticity in breast cancer cells. *Oncogene,* 24**,** 2375-85.

EL AMARI, E. B., TOUTOUS-TRELLU, L., GAYET-AGERON, A., BAUMANN, M., CATHOMAS, G., STEFFEN, I., ERB, P., MUELLER, N. J., FURRER, H., CAVASSINI, M., VERNAZZA, P., HIRSCH, H. H., BERNASCONI, E., HIRSCHEL, B. & SWISS, H. I. V. C. S. 2008. Predicting the evolution of Kaposi sarcoma, in the highly active antiretroviral therapy era. *AIDS,* 22**,** 1019-28.

ELKABETS, M., GIFFORD, A. M., SCHEEL, C., NILSSON, B., REINHARDT, F., BRAY, M. A., CARPENTER, A. E., JIRSTROM, K., MAGNUSSON, K., EBERT, B. L., PONTEN, F., WEINBERG, R. A. & MCALLISTER, S. S. 2011. Human tumors instigate granulin-expressing hematopoietic cells that promote malignancy by activating stromal fibroblasts in mice. *J Clin Invest,* 121**,** 784-99.

ELLIS, M., CHEW, Y. P., FALLIS, L., FREDDERSDORF, S., BOSHOFF, C., WEISS, R. A., LU, X. & MITTNACHT, S. 1999. Degradation of p27(Kip) cdk inhibitor triggered by Kaposi's sarcoma virus cyclin-cdk6 complex. *EMBO J,* 18**,** 644-53.

EMUSS, V., LAGOS, D., PIZZEY, A., GRATRIX, F., HENDERSON, S. R. & BOSHOFF, C. 2009. KSHV manipulates Notch signaling by DLL4 and JAG1 to alter cell cycle genes in lymphatic endothelia. *PLoS Pathog,* 5**,** e1000616.

ESPINOZA, I. & MIELE, L. 2013. Deadly crosstalk: Notch signaling at the intersection of EMT and cancer stem cells. *Cancer Lett,* 341**,** 41-5.

FAKHARI, F. D., JEONG, J. H., KANAN, Y. & DITTMER, D. P. 2006. The latency-associated nuclear antigen of Kaposi sarcoma-associated herpesvirus induces B cell hyperplasia and lymphoma. *J Clin Invest,* 116**,** 735-42.

FAN, X., KHAKI, L., ZHU, T. S., SOULES, M. E., TALSMA, C. E., GUL, N., KOH, C., ZHANG, J., LI, Y. M., MACIACZYK, J., NIKKHAH, G., DIMECO, F., PICCIRILLO, S., VESCOVI, A. L. & EBERHART, C. G. 2010. NOTCH pathway blockade depletes CD133-positive glioblastoma cells and inhibits growth of tumor neurospheres and xenografts. *Stem Cells,* 28**,** 5-16.

FERRANDO, A. A., NEUBERG, D. S., STAUNTON, J., LOH, M. L., HUARD, C., RAIMONDI, S. C., BEHM, F. G., PUI, C. H., DOWNING, J. R., GILLILAND, D. G., LANDER, E. S., GOLUB, T. R. & LOOK, A. T. 2002. Gene expression signatures define novel oncogenic pathways in T cell acute lymphoblastic leukemia. *Cancer Cell,* 1**,** 75-87.

FISCHER, A., SCHUMACHER, N., MAIER, M., SENDTNER, M. & GESSLER, M. 2004. The Notch target genes Hey1 and Hey2 are required for embryonic vascular development. *Genes Dev,* 18**,** 901-11.

FLISTER, M. J., WILBER, A., HALL, K. L., IWATA, C., MIYAZONO, K., NISATO, R. E., PEPPER, M. S., ZAWIEJA, D. C. & RAN, S. 2010. Inflammation induces lymphangiogenesis through up-regulation of VEGFR-3 mediated by NF-kappaB and Prox1. *Blood,* 115**,** 418-29.

FORTELNY, N., COX, J. H., KAPPELHOFF, R., STARR, A. E., LANGE, P. F., PAVLIDIS, P. & OVERALL, C. M. 2014. Network analyses reveal pervasive functional regulation between proteases in the human protease web. *PLoS Biol,* 12**,** e1001869.

FRIBORG, J., JR., KONG, W., HOTTIGER, M. O. & NABEL, G. J. 1999. p53 inhibition by the LANA protein of KSHV protects against cell death. *Nature,* 402**,** 889-94.

FRIDMAN, J. S. & LOWE, S. W. 2003. Control of apoptosis by p53. *Oncogene,* 22**,** 9030-40.

FRIEDL, P. & ALEXANDER, S. 2011. Cancer invasion and the microenvironment: plasticity and reciprocity. *Cell,* 147**,** 992-1009.

FRIEDL, P. & WOLF, K. 2009. Proteolytic interstitial cell migration: a five-step process. *Cancer Metastasis Rev,* 28**,** 129-35.

FRIEDMAN-KIEN, A. E. 1981. Disseminated Kaposi's sarcoma syndrome in young homosexual men. *J Am Acad Dermatol,* 5**,** 468-71.

GABAY, M., LI, Y. & FELSHER, D. W. 2014. MYC activation is a hallmark of cancer initiation and maintenance. *Cold Spring Harb Perspect Med,* 4.

GAIDANO, G., GLOGHINI, A., GATTEI, V., ROSSI, M. F., CILIA, A. M., GODEAS, C., DEGAN, M., PERIN, T., CANZONIERI, V., ALDINUCCI, D., SAGLIO, G., CARBONE, A. & PINTO, A. 1997. Association of Kaposi's sarcoma-associated herpesvirus-positive primary effusion lymphoma with expression of the CD138/syndecan-1 antigen. *Blood,* 90**,** 4894-900.

GAJEWSKI, T. F., SCHREIBER, H. & FU, Y. X. 2013. Innate and adaptive immune cells in the tumor microenvironment. *Nat Immunol,* 14**,** 1014-22.

GALON, J., COSTES, A., SANCHEZ-CABO, F., KIRILOVSKY, A., MLECNIK, B., LAGORCE-PAGES, C., TOSOLINI, M., CAMUS, M., BERGER, A., WIND, P., ZINZINDOHOUE, F., BRUNEVAL, P., CUGNENC, P. H., TRAJANOSKI, Z., FRIDMAN, W. H. & PAGES, F. 2006. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science,* 313**,** 1960-4.

GANEM, D. 2010. KSHV and the pathogenesis of Kaposi sarcoma: listening to human biology and medicine. *J Clin Invest,* 120**,** 939-49.

GARCIA-ROMAN, J. & ZENTELLA-DEHESA, A. 2013. Vascular permeability changes involved in tumor metastasis. *Cancer Lett,* 335**,** 259-69.

GARSIDE, V. C., CHANG, A. C., KARSAN, A. & HOODLESS, P. A. 2013. Co-ordinating Notch, BMP, and TGF-beta signaling during heart valve development. *Cell Mol Life Sci,* 70**,** 2899-917.

GASPERINI, P., ESPIGOL-FRIGOLE, G., MCCORMICK, P. J., SALVUCCI, O., MARIC, D., ULDRICK, T. S., POLIZZOTTO, M. N., YARCHOAN, R. & TOSATO, G. 2012. Kaposi sarcoma herpesvirus promotes endothelial-to-mesenchymal transition through Notch-dependent signaling. *Cancer Res,* 72**,** 1157-69.

GASPERINI, P., SAKAKIBARA, S. & TOSATO, G. 2008. Contribution of viral and cellular cytokines to Kaposi's sarcoma-associated herpesvirus pathogenesis. *J Leukoc Biol,* 84**,** 994-1000.

GAY, L. J. & FELDING-HABERMANN, B. 2011. Contribution of platelets to tumour metastasis. *Nat Rev Cancer,* 11**,** 123-34.

GERSHENGORN, M. C., GERAS-RAAKA, E., VARMA, A. & CLARK-LEWIS, I. 1998. Chemokines activate Kaposi's sarcoma-associated herpesvirus G protein-coupled receptor in mammalian cells in culture. *J Clin Invest,* 102**,** 1469-72.

GIFFIN, L. & DAMANIA, B. 2014. KSHV: pathways to tumorigenesis and persistent infection. *Adv Virus Res,* 88**,** 111-59.

GODDEN-KENT, D., TALBOT, S. J., BOSHOFF, C., CHANG, Y., MOORE, P., WEISS, R. A. & MITTNACHT, S. 1997. The cyclin encoded by Kaposi's sarcoma-associated herpesvirus stimulates cdk6 to phosphorylate the retinoblastoma protein and histone H1. *J Virol,* 71**,** 4193-8.

GODING, C. R., PEI, D. & LU, X. 2014. Cancer: pathological nuclear reprogramming? *Nat Rev Cancer,* 14**,** 568-73.

GONZALEZ, D. M. & MEDICI, D. 2014. Signaling mechanisms of the epithelial-mesenchymal transition. *Sci Signal,* 7**,** re8.

GORITZ, C., DIAS, D. O., TOMILIN, N., BARBACID, M., SHUPLIAKOV, O. & FRISEN, J. 2011. A pericyte origin of spinal cord scar tissue. *Science,* 333**,** 238-42.

GOSWAMI, S., SAHAI, E., WYCKOFF, J. B., CAMMER, M., COX, D., PIXLEY, F. J., STANLEY, E. R., SEGALL, J. E. & CONDEELIS, J. S. 2005. Macrophages promote the invasion of breast carcinoma cells via a colony-stimulating factor-1/epidermal growth factor paracrine loop. *Cancer Res,* 65**,** 5278-83.

GOTO, H., KOJIMA, Y., NAGAI, H. & OKADA, S. 2013. Establishment of a CD4-positive cell line from an AIDS-related primary effusion lymphoma. *Int J Hematol,* 97**,** 624-33.

GREEN, J. L., LA, J., YUM, K. W., DESAI, P., RODEWALD, L. W., ZHANG, X., LEBLANC, M., NUSSE, R., LEWIS, M. T. & WAHL, G. M. 2013. Paracrine Wnt signaling both promotes and inhibits human breast tumor growth. *Proc Natl Acad Sci U S A,* 110**,** 6991-6.

GRIVENNIKOV, S. I. & KARIN, M. 2010. Dangerous liaisons: STAT3 and NF-kappaB collaboration and crosstalk in cancer. *Cytokine Growth Factor Rev,* 21**,** 11-9.

GROSSEL, M. J. & HINDS, P. W. 2006a. Beyond the cell cycle: a new role for Cdk6 in differentiation. *J Cell Biochem,* 97**,** 485-93.

GROSSEL, M. J. & HINDS, P. W. 2006b. From cell cycle to differentiation: an expanding role for cdk6. *Cell Cycle,* 5**,** 266-70.

GROSSMANN, C., PODGRABINSKA, S., SKOBE, M. & GANEM, D. 2006. Activation of NF-kappaB by the latent vFLIP gene of Kaposi's sarcoma-associated herpesvirus is required for the spindle shape of virus-infected endothelial cells and contributes to their proinflammatory phenotype. *J Virol,* 80**,** 7179-85.

GRUNDHOFF, A. & GANEM, D. 2004. Inefficient establishment of KSHV latency suggests an additional role for continued lytic replication in Kaposi sarcoma pathogenesis. *J Clin Invest,* 113**,** 124-36.

GUASPARRI, I., KELLER, S. A. & CESARMAN, E. 2004. KSHV vFLIP is essential for the survival of infected lymphoma cells. *J Exp Med,* 199**,** 993-1003.

GUASPARRI, I., WU, H. & CESARMAN, E. 2006. The KSHV oncoprotein vFLIP contains a TRAF-interacting motif and requires TRAF2 and TRAF3 for signalling. *EMBO Rep,* 7**,** 114-9.

GURUHARSHA, K. G., KANKEL, M. W. & ARTAVANIS-TSAKONAS, S. 2012. The Notch signalling system: recent insights into the complexity of a conserved pathway. *Nat Rev Genet,* 13**,** 654-66.

GUTIERREZ, K. D., MORRIS, V. A., WU, D., BARCY, S. & LAGUNOFF, M. 2013. Ets-1 is required for the activation of VEGFR3 during latent Kaposi's sarcoma-associated herpesvirus infection of endothelial cells. *J Virol,* 87**,** 6758-68.

GUTTRIDGE, D. C., ALBANESE, C., REUTHER, J. Y., PESTELL, R. G. & BALDWIN, A. S., JR. 1999. NF-kappaB controls cell growth and differentiation through transcriptional regulation of cyclin D1. *Mol Cell Biol,* 19**,** 5785-99.

HAEGER, A., KRAUSE, M., WOLF, K. & FRIEDL, P. 2014. Cell jamming: collective invasion of mesenchymal tumor cells imposed by tissue confinement. *Biochim Biophys Acta,* 1840**,** 2386-95.

HAGEMANN, T., LAWRENCE, T., MCNEISH, I., CHARLES, K. A., KULBE, H., THOMPSON, R. G., ROBINSON, S. C. & BALKWILL, F. R. 2008. "Re-educating" tumor-associated macrophages by targeting NF-kappaB. *J Exp Med,* 205**,** 1261-8.

HAMIDI, H., GUSTAFASON, D., PELLEGRINI, M. & GASSON, J. 2011. Identification of novel targets of CSL-dependent Notch signaling in hematopoiesis. *PLoS One,* 6**,** e20022.

HANAHAN, D. & FOLKMAN, J. 1996. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell,* 86**,** 353-64.

HANAHAN, D. & WEINBERG, R. A. 2000. The hallmarks of cancer. *Cell,* 100**,** 57-70.

HANAHAN, D. & WEINBERG, R. A. 2011. Hallmarks of cancer: the next generation. *Cell,* 144**,** 646-74.

HARDY, K. M., KIRSCHMANN, D. A., SEFTOR, E. A., MARGARYAN, N. V., POSTOVIT, L. M., STRIZZI, L. & HENDRIX, M. J. 2010. Regulation of the embryonic morphogen Nodal by Notch4 facilitates manifestation of the aggressive melanoma phenotype. *Cancer Res,* 70**,** 10340-50.

HASSMAN, L. M., ELLISON, T. J. & KEDES, D. H. 2011. KSHV infects a subset of human tonsillar B cells, driving proliferation and plasmablast differentiation. *J Clin Invest,* 121**,** 752-68.

HAYDEN, M. S. & GHOSH, S. 2008. Shared principles in NF-kappaB signaling. *Cell,* 132**,** 344-62.

HE, Y., KOZAKI, K., KARPANEN, T., KOSHIKAWA, K., YLA-HERTTUALA, S., TAKAHASHI, T. & ALITALO, K. 2002. Suppression of tumor lymphangiogenesis and lymph node metastasis by blocking vascular endothelial growth factor receptor 3 signaling. *J Natl Cancer Inst,* 94**,** 819-25.

HELDIN, C. H., VANLANDEWIJCK, M. & MOUSTAKAS, A. 2012. Regulation of EMT by TGFbeta in cancer. *FEBS Lett,* 586**,** 1959-70.

HENDRIX, M. J., SEFTOR, E. A., HESS, A. R. & SEFTOR, R. E. 2003. Molecular plasticity of human melanoma cells. *Oncogene,* 22**,** 3070-5.

HENDRIX, M. J., SEFTOR, E. A., SEFTOR, R. E., KASEMEIER-KULESA, J., KULESA, P. M. & POSTOVIT, L. M. 2007. Reprogramming metastatic tumour cells with embryonic microenvironments. *Nat Rev Cancer,* 7**,** 246-55.

HIGGINS, D. F., KIMURA, K., BERNHARDT, W. M., SHRIMANKER, N., AKAI, Y., HOHENSTEIN, B., SAITO, Y., JOHNSON, R. S., KRETZLER, M., COHEN, C. D., ECKARDT, K. U., IWANO, M. & HAASE, V. H. 2007. Hypoxia promotes fibrogenesis in vivo via HIF-1 stimulation of epithelial-to-mesenchymal transition. *J Clin Invest,* 117**,** 3810-20.

HIRAKAWA, S., BROWN, L. F., KODAMA, S., PAAVONEN, K., ALITALO, K. & DETMAR, M. 2007. VEGF-C-induced lymphangiogenesis in sentinel lymph nodes promotes tumor metastasis to distant sites. *Blood,* 109**,** 1010-7.

HIRAKAWA, S., DETMAR, M., KERJASCHKI, D., NAGAMATSU, S., MATSUO, K., TANEMURA, A., KAMATA, N., HIGASHIKAWA, K., OKAZAKI, H., KAMEDA, K., NISHIDA-FUKUDA, H., MORI, H., HANAKAWA, Y., SAYAMA, K., SHIRAKATA, Y., TOHYAMA, M., TOKUMARU, S., KATAYAMA, I. & HASHIMOTO, K. 2009. Nodal lymphangiogenesis and metastasis: Role of tumor-induced lymphatic vessel activation in extramammary Paget's disease. *Am J Pathol,* 175**,** 2235-48.

HIRAKAWA, S., KODAMA, S., KUNSTFELD, R., KAJIYA, K., BROWN, L. F. & DETMAR, M. 2005. VEGF-A induces tumor and sentinel lymph node lymphangiogenesis and promotes lymphatic metastasis. *J Exp Med,* 201**,** 1089-99.

HOESEL, B. & SCHMID, J. A. 2013. The complexity of NF-kappaB signaling in inflammation and cancer. *Mol Cancer,* 12**,** 86.

HONG, Y. K., FOREMAN, K., SHIN, J. W., HIRAKAWA, S., CURRY, C. L., SAGE, D. R., LIBERMANN, T., DEZUBE, B. J., FINGEROTH, J. D. & DETMAR, M. 2004. Lymphatic reprogramming of blood vascular endothelium by Kaposi sarcoma-associated herpesvirus. *Nat Genet,* 36**,** 683-5.

HOVINGA, K. E., SHIMIZU, F., WANG, R., PANAGIOTAKOS, G., VAN DER HEIJDEN, M., MOAYEDPARDAZI, H., CORREIA, A. S., SOULET, D., MAJOR, T., MENON, J. & TABAR, V. 2010. Inhibition of notch signaling in glioblastoma targets cancer stem cells via an endothelial cell intermediate. *Stem Cells,* 28**,** 1019-29.

HOWARD, J. D., MORIARTY, W. F., PARK, J., RIEDY, K., PANOVA, I. P., CHUNG, C. H., SUH, K. Y., LEVCHENKO, A. & ALANI, R. M. 2013. Notch signaling mediates melanoma-endothelial cell communication and melanoma cell migration. *Pigment Cell Melanoma Res,* 26**,** 697-707.

HU, J., GARBER, A. C. & RENNE, R. 2002. The latency-associated nuclear antigen of Kaposi's sarcoma-associated herpesvirus supports latent DNA replication in dividing cells. *J Virol,* 76**,** 11677-87.

HU, J., SRIVASTAVA, K., WIELAND, M., RUNGE, A., MOGLER, C., BESEMFELDER, E., TERHARDT, D., VOGEL, M. J., CAO, L., KORN, C., BARTELS, S., THOMAS, M. & AUGUSTIN, H. G. 2014. Endothelial cell-derived angiopoietin-2 controls liver regeneration as a spatiotemporal rheostat. *Science,* 343**,** 416-9.

HU, M. G., DESHPANDE, A., SCHLICHTING, N., HINDS, E. A., MAO, C., DOSE, M., HU, G. F., VAN ETTEN, R. A., GOUNARI, F. & HINDS, P. W. 2011. CDK6 kinase activity is required for thymocyte development. *Blood,* 117**,** 6120-31.

HUBER, M. A., AZOITEI, N., BAUMANN, B., GRUNERT, S., SOMMER, A., PEHAMBERGER, H., KRAUT, N., BEUG, H. & WIRTH, T. 2004. NF-kappaB is essential for epithelial-mesenchymal transition and metastasis in a model of breast cancer progression. *J Clin Invest,* 114**,** 569-81.

HUSEMANN, Y., GEIGL, J. B., SCHUBERT, F., MUSIANI, P., MEYER, M., BURGHART, E., FORNI, G., EILS, R., FEHM, T., RIETHMULLER, G. & KLEIN, C. A. 2008. Systemic spread is an early step in breast cancer. *Cancer Cell,* 13**,** 58-68.

IRJALA, H., ALANEN, K., GRENMAN, R., HEIKKILA, P., JOENSUU, H. & JALKANEN, S. 2003. Mannose receptor (MR) and common lymphatic endothelial and vascular endothelial receptor (CLEVER)-1 direct the binding of cancer cells to the lymph vessel endothelium. *Cancer Res,* 63**,** 4671-6.

IZON, D. J., PUNT, J. A., XU, L., KARNELL, F. G., ALLMAN, D., MYUNG, P. S., BOERTH, N. J., PUI, J. C., KORETZKY, G. A. & PEAR, W. S. 2001. Notch1 regulates maturation of CD4+ and CD8+ thymocytes by modulating TCR signal strength. *Immunity,* 14**,** 253-64.

JAKOBSSON, L., FRANCO, C. A., BENTLEY, K., COLLINS, R. T., PONSIOEN, B., ASPALTER, I. M., ROSEWELL, I., BUSSE, M., THURSTON, G., MEDVINSKY, A., SCHULTE-MERKER, S. & GERHARDT, H. 2010. Endothelial cells dynamically compete for the tip cell position during angiogenic sprouting. *Nat Cell Biol,* 12**,** 943-53.

JARVILUOMA, A. & OJALA, P. M. 2006. Cell signaling pathways engaged by KSHV. *Biochim Biophys Acta,* 1766**,** 140-58.

JHAM, B. C. & MONTANER, S. 2010. The Kaposi's sarcoma-associated herpesvirus G protein-coupled receptor: Lessons on dysregulated angiogenesis from a viral oncogene. *J Cell Biochem,* 110**,** 1-9.

JI, R. C. 2012. Macrophages are important mediators of either tumor- or inflammation-induced lymphangiogenesis. *Cell Mol Life Sci,* 69**,** 897-914.

JI, R. C. 2014. Hypoxia and lymphangiogenesis in tumor microenvironment and metastasis. *Cancer Lett,* 346**,** 6-16.

JIANG, J., TANG, Y. L. & LIANG, X. H. 2011. EMT: a new vision of hypoxia promoting cancer progression. *Cancer Biol Ther,* 11**,** 714-23.

JIN, S., HANSSON, E. M., TIKKA, S., LANNER, F., SAHLGREN, C., FARNEBO, F., BAUMANN, M., KALIMO, H. & LENDAHL, U. 2008. Notch signaling regulates platelet-derived growth factor receptor-beta expression in vascular smooth muscle cells. *Circ Res,* 102**,** 1483-91.

JOHNSON, N. C., DILLARD, M. E., BALUK, P., MCDONALD, D. M., HARVEY, N. L., FRASE, S. L. & OLIVER, G. 2008. Lymphatic endothelial cell identity is reversible and its maintenance requires Prox1 activity. *Genes Dev,* 22**,** 3282-91.

JONES, T., RAMOS DA SILVA, S., BEDOLLA, R., YE, F., ZHOU, F. & GAO, S. J. 2014. Viral cyclin promotes KSHV-induced cellular transformation and tumorigenesis by overriding contact inhibition. *Cell Cycle,* 13.

JONES, T., YE, F., BEDOLLA, R., HUANG, Y., MENG, J., QIAN, L., PAN, H., ZHOU, F., MOODY, R., WAGNER, B., ARAR, M. & GAO, S. J. 2012. Direct and efficient cellular transformation of primary rat mesenchymal precursor cells by KSHV. *J Clin Invest,* 122**,** 1076-81.

JOSHI, I., MINTER, L. M., TELFER, J., DEMAREST, R. M., CAPOBIANCO, A. J., ASTER, J. C., SICINSKI, P., FAUQ, A., GOLDE, T. E. & OSBORNE, B. A. 2009. Notch signaling mediates G1/S cell-cycle progression in T cells via cyclin D3 and its dependent kinases. *Blood,* 113**,** 1689-98.

JUNCKER-JENSEN, A., DERYUGINA, E. I., RIMANN, I., ZAJAC, E., KUPRIYANOVA, T. A., ENGELHOLM, L. H. & QUIGLEY, J. P. 2013. Tumor MMP-1 activates endothelial PAR1 to facilitate vascular intravasation and metastatic dissemination. *Cancer Res,* 73**,** 4196-211.

JUSSILA, L., VALTOLA, R., PARTANEN, T. A., SALVEN, P., HEIKKILA, P., MATIKAINEN, M. T., RENKONEN, R., KAIPAINEN, A., DETMAR, M., TSCHACHLER, E., ALITALO, R. & ALITALO, K. 1998. Lymphatic endothelium and Kaposi's sarcoma spindle cells detected by antibodies against the vascular endothelial growth factor receptor-3. *Cancer Res,* 58**,** 1599-604.

KAAYA, E. E., PARRAVICINI, C., ORDONEZ, C., GENDELMAN, R., BERTI, E., GALLO, R. C. & BIBERFELD, P. 1995. Heterogeneity of spindle cells in Kaposi's sarcoma: comparison of cells in lesions and in culture. *J Acquir Immune Defic Syndr Hum Retrovirol,* 10**,** 295-305.

KAKINUMA, T. & HWANG, S. T. 2006. Chemokines, chemokine receptors, and cancer metastasis. *J Leukoc Biol,* 79**,** 639-51.

KALLURI, R. & WEINBERG, R. A. 2009. The basics of epithelial-mesenchymal transition. *J Clin Invest,* 119**,** 1420-8.

KARAMAN, S. & DETMAR, M. 2014. Mechanisms of lymphatic metastasis. *J Clin Invest,* 124**,** 922-8.

KARIKOSKI, M., MARTTILA-ICHIHARA, F., ELIMA, K., RANTAKARI, P., HOLLMEN, M., KELKKA, T., GERKE, H., HUOVINEN, V., IRJALA, H., HOLMDAHL, R., SALMI, M. & JALKANEN, S. 2014. Clever-1/stabilin-1 controls cancer growth and metastasis. *Clin Cancer Res,* 20**,** 6452-64.

KARPANEN, T., EGEBLAD, M., KARKKAINEN, M. J., KUBO, H., YLA-HERTTUALA, S., JAATTELA, M. & ALITALO, K. 2001. Vascular endothelial growth factor C promotes tumor lymphangiogenesis and intralymphatic tumor growth. *Cancer Res,* 61**,** 1786-90.

KATOH, Y. & KATOH, M. 2009. FGFR2-related pathogenesis and FGFR2-targeted therapeutics (Review). *Int J Mol Med,* 23**,** 307-11.

KELLER, S. A., SCHATTNER, E. J. & CESARMAN, E. 2000. Inhibition of NF-kappaB induces apoptosis of KSHV-infected primary effusion lymphoma cells. *Blood,* 96**,** 2537-42.

KERJASCHKI, D., BAGO-HORVATH, Z., RUDAS, M., SEXL, V., SCHNECKENLEITHNER, C., WOLBANK, S., BARTEL, G., KRIEGER, S., KALT, R., HANTUSCH, B., KELLER, T., NAGY-BOJARSZKY, K., HUTTARY, N., RAAB, I., LACKNER, K., KRAUTGASSER, K., SCHACHNER, H., KASERER, K., REZAR, S., MADLENER, S., VONACH, C., DAVIDOVITS, A., NOSAKA, H., HAMMERLE, M., VIOLA, K., DOLZNIG, H., SCHREIBER, M., NADER, A., MIKULITS, W., GNANT, M., HIRAKAWA, S., DETMAR, M., ALITALO, K., NIJMAN, S., OFFNER, F., MAIER, T. J., STEINHILBER, D. & KRUPITZA, G. 2011. Lipoxygenase mediates invasion of intrametastatic lymphatic vessels and propagates lymph node metastasis of human mammary carcinoma xenografts in mouse. *J Clin Invest,* 121**,** 2000-12.

KESSENBROCK, K., PLAKS, V. & WERB, Z. 2010. Matrix metalloproteinases: regulators of the tumor microenvironment. *Cell,* 141**,** 52-67.

KIM, M., KOH, Y. J., KIM, K. E., KOH, B. I., NAM, D. H., ALITALO, K., KIM, I. & KOH, G. Y. 2010. CXCR4 signaling regulates metastasis of chemoresistant melanoma cells by a lymphatic metastatic niche. *Cancer Res,* 70**,** 10411-21.

KLEIN, C. A. 2009. Parallel progression of primary tumours and metastases. *Nat Rev Cancer,* 9**,** 302-12.

KLEIN, C. A. & HOLZEL, D. 2006. Systemic cancer progression and tumor dormancy: mathematical models meet single cell genomics. *Cell Cycle,* 5**,** 1788-98.

KLEIN, U., GLOGHINI, A., GAIDANO, G., CHADBURN, A., CESARMAN, E., DALLA-FAVERA, R. & CARBONE, A. 2003. Gene expression profile analysis of AIDS-related primary effusion lymphoma (PEL) suggests a plasmablastic derivation and identifies PEL-specific transcripts. *Blood,* 101**,** 4115-21.

KOCH, S., TUGUES, S., LI, X., GUALANDI, L. & CLAESSON-WELSH, L. 2011. Signal transduction by vascular endothelial growth factor receptors. *Biochem J,* 437**,** 169-83.

KOCH, U. & RADTKE, F. 2007. Notch and cancer: a double-edged sword. *Cell Mol Life Sci,* 64**,** 2746-62.

KOFLER, N. M., SHAWBER, C. J., KANGSAMAKSIN, T., REED, H. O., GALATIOTO, J. & KITAJEWSKI, J. 2011. Notch signaling in developmental and tumor angiogenesis. *Genes Cancer,* 2**,** 1106-16.

KOOPAL, S., FURUHJELM, J. H., JARVILUOMA, A., JAAMAA, S., PYAKUREL, P., PUSSINEN, C., WIRZENIUS, M., BIBERFELD, P., ALITALO, K., LAIHO, M. & OJALA, P. M. 2007. Viral oncogene-induced DNA damage response is activated in Kaposi sarcoma tumorigenesis. *PLoS Pathog,* 3**,** 1348-60.

KORFF, T. & AUGUSTIN, H. G. 1998. Integration of endothelial cells in multicellular spheroids prevents apoptosis and induces differentiation. *J Cell Biol,* 143**,** 1341-52.

KREBS, L. T., SHUTTER, J. R., TANIGAKI, K., HONJO, T., STARK, K. L. & GRIDLEY, T. 2004. Haploinsufficient lethality and formation of arteriovenous malformations in Notch pathway mutants. *Genes Dev,* 18**,** 2469-73.

KREBS, L. T., XUE, Y., NORTON, C. R., SHUTTER, J. R., MAGUIRE, M., SUNDBERG, J. P., GALLAHAN, D., CLOSSON, V., KITAJEWSKI, J., CALLAHAN, R., SMITH, G. H., STARK, K. L. & GRIDLEY, T. 2000. Notch signaling is essential for vascular morphogenesis in mice. *Genes Dev,* 14**,** 1343-52.

KUSHWAH, R., GUEZGUEZ, B., LEE, J. B., HOPKINS, C. I. & BHATIA, M. 2014. Pleiotropic roles of Notch signaling in normal, malignant, and developmental hematopoiesis in the human. *EMBO Rep,* 15**,** 1128-1138.

LA FERLA, L., PINZONE, M. R., NUNNARI, G., MARTELLOTTA, F., LLESHI, A., TIRELLI, U., DE PAOLI, P., BERRETTA, M. & CACOPARDO, B. 2013. Kaposi' s sarcoma in HIV-positive patients: the state of art in the HAART-era. *Eur Rev Med Pharmacol Sci,* 17**,** 2354-65.

LA ROSA, F. A., PIERCE, J. W. & SONENSHEIN, G. E. 1994. Differential regulation of the c-myc oncogene promoter by the NF-kappa B rel family of transcription factors. *Mol Cell Biol,* 14**,** 1039-44.

LAGUNOFF, M., BECHTEL, J., VENETSANAKOS, E., ROY, A. M., ABBEY, N., HERNDIER, B., MCMAHON, M. & GANEM, D. 2002. De novo infection and serial transmission of Kaposi's sarcoma-associated herpesvirus in cultured endothelial cells. *J Virol,* 76**,** 2440-8.

LAMAN, H., COVERLEY, D., KRUDE, T., LASKEY, R. & JONES, N. 2001. Viral cyclin-cyclin-dependent kinase 6 complexes initiate nuclear DNA replication. *Mol Cell Biol,* 21**,** 624-35.

LAN, K., CHOUDHURI, T., MURAKAMI, M., KUPPERS, D. A. & ROBERTSON, E. S. 2006. Intracellular activated Notch1 is critical for proliferation of Kaposi's sarcoma-associated herpesvirus-associated B-lymphoma cell lines in vitro. *J Virol,* 80**,** 6411-9.

LAN, K., MURAKAMI, M., BAJAJ, B., KAUL, R., HE, Z., GAN, R., FELDMAN, M. & ROBERTSON, E. S. 2009. Inhibition of KSHV-infected primary effusion lymphomas in NOD/SCID mice by gamma-secretase inhibitor. *Cancer Biol Ther,* 8**,** 2136-43.

LAN, K., VERMA, S. C., MURAKAMI, M., BAJAJ, B., KAUL, R. & ROBERTSON, E. S. 2007. Kaposi's sarcoma herpesvirus-encoded latency-associated nuclear antigen stabilizes intracellular activated Notch by targeting the Sel10 protein. *Proc Natl Acad Sci U S A,* 104**,** 16287-92.

LANDIS, M. W., PAWLYK, B. S., LI, T., SICINSKI, P. & HINDS, P. W. 2006. Cyclin D1-dependent kinase activity in murine development and mammary tumorigenesis. *Cancer Cell,* 9**,** 13-22.

LARKIN, J., ASCIERTO, P. A., DRENO, B., ATKINSON, V., LISZKAY, G., MAIO, M., MANDALA, M., DEMIDOV, L., STROYAKOVSKIY, D., THOMAS, L., DE LA CRUZ-MERINO, L., DUTRIAUX, C., GARBE, C., SOVAK, M. A., CHANG, I., CHOONG, N., HACK, S. P., MCARTHUR, G. A. & RIBAS, A. 2014. Combined Vemurafenib and Cobimetinib in BRAF-Mutated Melanoma. *N Engl J Med*.

LAWRENCE, T., BEBIEN, M., LIU, G. Y., NIZET, V. & KARIN, M. 2005. IKKalpha limits macrophage NF-kappaB activation and contributes to the resolution of inflammation. *Nature,* 434**,** 1138-43.

LEE, E., FERTIG, E. J., JIN, K., SUKUMAR, S., PANDEY, N. B. & POPEL, A. S. 2014a. Breast cancer cells condition lymphatic endothelial cells within pre-metastatic niches to promote metastasis. *Nat Commun,* 5**,** 4715.

LEE, E., PANDEY, N. B. & POPEL, A. S. 2014b. Pre-treatment of mice with tumor-conditioned media accelerates metastasis to lymph nodes and lungs: a new spontaneous breast cancer metastasis model. *Clin Exp Metastasis,* 31**,** 67-79.

LEE, J. S., LI, Q., LEE, J. Y., LEE, S. H., JEONG, J. H., LEE, H. R., CHANG, H., ZHOU, F. C., GAO, S. J., LIANG, C. & JUNG, J. U. 2009. FLIP-mediated autophagy regulation in cell death control. *Nat Cell Biol,* 11**,** 1355-62.

LEFORT, N., BENNE, C., LELIEVRE, J. D., DORIVAL, C., BALBO, M., SAKANO, S., COULOMBEL, L. & LEVY, Y. 2006. Short exposure to Notch ligand Delta-4 is sufficient to induce T-cell differentiation program and to increase the T cell potential of primary human CD34+ cells. *Exp Hematol,* 34**,** 1720-9.

LEIDAL, A. M., CYR, D. P., HILL, R. J., LEE, P. W. & MCCORMICK, C. 2012. Subversion of autophagy by Kaposi's sarcoma-associated herpesvirus impairs oncogene-induced senescence. *Cell Host Microbe,* 11**,** 167-80.

LEONG, K. G., NIESSEN, K., KULIC, I., RAOUF, A., EAVES, C., POLLET, I. & KARSAN, A. 2007. Jagged1-mediated Notch activation induces epithelial-to-mesenchymal transition through Slug-induced repression of E-cadherin. *J Exp Med,* 204**,** 2935-48.

LIU, H., KENNARD, S. & LILLY, B. 2009. NOTCH3 expression is induced in mural cells through an autoregulatory loop that requires endothelial-expressed JAGGED1. *Circ Res,* 104**,** 466-75.

LIU, H., ZHANG, W., KENNARD, S., CALDWELL, R. B. & LILLY, B. 2010. Notch3 is critical for proper angiogenesis and mural cell investment. *Circ Res,* 107**,** 860-70.

LIU, L., EBY, M. T., RATHORE, N., SINHA, S. K., KUMAR, A. & CHAUDHARY, P. M. 2002. The human herpes virus 8-encoded viral FLICE inhibitory protein physically associates with and persistently activates the Ikappa B kinase complex. *J Biol Chem,* 277**,** 13745-51.

LIU, Z. J., XIAO, M., BALINT, K., SMALLEY, K. S., BRAFFORD, P., QIU, R., PINNIX, C. C., LI, X. & HERLYN, M. 2006. Notch1 signaling promotes primary melanoma progression by activating mitogen-activated protein kinase/phosphatidylinositol 3-kinase-Akt pathways and up-regulating N-cadherin expression. *Cancer Res,* 66**,** 4182-90.

LOBRY, C., OH, P., MANSOUR, M. R., LOOK, A. T. & AIFANTIS, I. 2014. Notch signaling: switching an oncogene to a tumor suppressor. *Blood,* 123**,** 2451-9.

MA, L., LU, M. F., SCHWARTZ, R. J. & MARTIN, J. F. 2005. Bmp2 is essential for cardiac cushion epithelial-mesenchymal transition and myocardial patterning. *Development,* 132**,** 5601-11.

MA, T., JHAM, B. C., HU, J., FRIEDMAN, E. R., BASILE, J. R., MOLINOLO, A., SODHI, A. & MONTANER, S. 2010. Viral G protein-coupled receptor up-regulates Angiopoietin-like 4 promoting angiogenesis and vascular permeability in Kaposi's sarcoma. *Proc Natl Acad Sci U S A,* 107**,** 14363-8.

MADAR, S., GOLDSTEIN, I. & ROTTER, V. 2013. 'Cancer associated fibroblasts'--more than meets the eye. *Trends Mol Med,* 19**,** 447-53.

MAKINEN, T., VEIKKOLA, T., MUSTJOKI, S., KARPANEN, T., CATIMEL, B., NICE, E. C., WISE, L., MERCER, A., KOWALSKI, H., KERJASCHKI, D., STACKER, S. A., ACHEN, M. G. & ALITALO, K. 2001. Isolated lymphatic endothelial cells transduce growth, survival and migratory signals via the VEGF-C/D receptor VEGFR-3. *EMBO J,* 20**,** 4762-73.

MALANCHI, I., SANTAMARIA-MARTINEZ, A., SUSANTO, E., PENG, H., LEHR, H. A., DELALOYE, J. F. & HUELSKEN, J. 2012. Interactions between cancer stem cells and their niche govern metastatic colonization. *Nature,* 481**,** 85-9.

MALUMBRES, M. & BARBACID, M. 2001. To cycle or not to cycle: a critical decision in cancer. *Nat Rev Cancer,* 1**,** 222-31.

MALUMBRES, M. & BARBACID, M. 2009. Cell cycle, CDKs and cancer: a changing paradigm. *Nat Rev Cancer,* 9**,** 153-66.

MANDRIOTA, S. J., JUSSILA, L., JELTSCH, M., COMPAGNI, A., BAETENS, D., PREVO, R., BANERJI, S., HUARTE, J., MONTESANO, R., JACKSON, D. G., ORCI, L., ALITALO, K., CHRISTOFORI, G. & PEPPER, M. S. 2001. Vascular endothelial growth factor-C-mediated lymphangiogenesis promotes tumour metastasis. *EMBO J,* 20**,** 672-82.

MANN, D. J., CHILD, E. S., SWANTON, C., LAMAN, H. & JONES, N. 1999. Modulation of p27(Kip1) levels by the cyclin encoded by Kaposi's sarcoma-associated herpesvirus. *EMBO J,* 18**,** 654-63.

MANSOURI, M., DOUGLAS, J., ROSE, P. P., GOUVEIA, K., THOMAS, G., MEANS, R. E., MOSES, A. V. & FRUH, K. 2006. Kaposi sarcoma herpesvirus K5 removes CD31/PECAM from endothelial cells. *Blood,* 108**,** 1932-40.

MASOOD, R., CAI, J., ZHENG, T., SMITH, D. L., NAIDU, Y. & GILL, P. S. 1997. Vascular endothelial growth factor/vascular permeability factor is an autocrine growth factor for AIDS-Kaposi sarcoma. *Proc Natl Acad Sci U S A,* 94**,** 979-84.

MAY, M. J. & GHOSH, S. 1997. Rel/NF-kappa B and I kappa B proteins: an overview. *Semin Cancer Biol,* 8**,** 63-73.

MCALLISTER, S. C. & MOSES, A. V. 2007. Endothelial cell- and lymphocyte-based in vitro systems for understanding KSHV biology. *Curr Top Microbiol Immunol,* 312**,** 211-44.

MCARTHUR, G. A., CHAPMAN, P. B., ROBERT, C., LARKIN, J., HAANEN, J. B., DUMMER, R., RIBAS, A., HOGG, D., HAMID, O., ASCIERTO, P. A., GARBE, C., TESTORI, A., MAIO, M., LORIGAN, P., LEBBE, C., JOUARY, T., SCHADENDORF, D., O'DAY, S. J., KIRKWOOD, J. M., EGGERMONT, A. M., DRENO, B., SOSMAN, J. A., FLAHERTY, K. T., YIN, M., CARO, I., CHENG, S., TRUNZER, K. & HAUSCHILD, A. 2014. Safety and efficacy of vemurafenib in BRAF(V600E) and BRAF(V600K) mutation-positive melanoma (BRIM-3): extended follow-up of a phase 3, randomised, open-label study. *Lancet Oncol,* 15**,** 323-32.

MCCORMACK, N. & O'DEA, S. 2013. Regulation of epithelial to mesenchymal transition by bone morphogenetic proteins. *Cell Signal,* 25**,** 2856-62.

MCCULLEY, D. J., KANG, J. O., MARTIN, J. F. & BLACK, B. L. 2008. BMP4 is required in the anterior heart field and its derivatives for endocardial cushion remodeling, outflow tract septation, and semilunar valve development. *Dev Dyn,* 237**,** 3200-9.

MEDICI, D. & KALLURI, R. 2012. Endothelial-mesenchymal transition and its contribution to the emergence of stem cell phenotype. *Semin Cancer Biol,* 22**,** 379-84.

MEDICI, D. & OLSEN, B. R. 2012. The role of endothelial-mesenchymal transition in heterotopic ossification. *J Bone Miner Res,* 27**,** 1619-22.

MEDICI, D., POTENTA, S. & KALLURI, R. 2011. Transforming growth factor-beta2 promotes Snail-mediated endothelial-mesenchymal transition through convergence of Smad-dependent and Smad-independent signalling. *Biochem J,* 437**,** 515-20.

MEDICI, D., SHORE, E. M., LOUNEV, V. Y., KAPLAN, F. S., KALLURI, R. & OLSEN, B. R. 2010. Conversion of vascular endothelial cells into multipotent stem-like cells. *Nat Med,* 16**,** 1400-6.

MESRI, E. A., FEITELSON, M. A. & MUNGER, K. 2014. Human viral oncogenesis: a cancer hallmarks analysis. *Cell Host Microbe,* 15**,** 266-82.

MEUWISSEN, R. & BERNS, A. 2005. Mouse models for human lung cancer. *Genes Dev,* 19**,** 643-64.

MIRANDOLA, L., APICELLA, L., COLOMBO, M., YU, Y., BERTA, D. G., PLATONOVA, N., LAZZARI, E., LANCELLOTTI, M., BULFAMANTE, G., COBOS, E., CHIRIVA-INTERNATI, M. & CHIARAMONTE, R. 2013. Anti-Notch treatment prevents multiple myeloma cells localization to the bone marrow via the chemokine system CXCR4/SDF-1. *Leukemia,* 27**,** 1558-66.

MIYAZONO, K. 2009. Transforming growth factor-beta signaling in epithelial-mesenchymal transition and progression of cancer. *Proc Jpn Acad Ser B Phys Biol Sci,* 85**,** 314-23.

MIYOSHI, A., KITAJIMA, Y., SUMI, K., SATO, K., HAGIWARA, A., KOGA, Y. & MIYAZAKI, K. 2004. Snail and SIP1 increase cancer invasion by upregulating MMP family in hepatocellular carcinoma cells. *Br J Cancer,* 90**,** 1265-73.

MOBLEY, A. K., BRAEUER, R. R., KAMIYA, T., SHOSHAN, E. & BAR-ELI, M. 2012. Driving transcriptional regulators in melanoma metastasis. *Cancer Metastasis Rev,* 31**,** 621-32.

MONTANER, S. 2007. Akt/TSC/mTOR activation by the KSHV G protein-coupled receptor: emerging insights into the molecular oncogenesis and treatment of Kaposi's sarcoma. *Cell Cycle,* 6**,** 438-43.

MONTANER, S., SODHI, A., MOLINOLO, A., BUGGE, T. H., SAWAI, E. T., HE, Y., LI, Y., RAY, P. E. & GUTKIND, J. S. 2003. Endothelial infection with KSHV genes in vivo reveals that vGPCR initiates Kaposi's sarcomagenesis and can promote the tumorigenic potential of viral latent genes. *Cancer Cell,* 3**,** 23-36.

MONTANER, S., SODHI, A., PECE, S., MESRI, E. A. & GUTKIND, J. S. 2001. The Kaposi's sarcoma-associated herpesvirus G protein-coupled receptor promotes endothelial cell survival through the activation of Akt/protein kinase B. *Cancer Res,* 61**,** 2641-8.

MOORE, P. S. & CHANG, Y. 1995. Detection of herpesvirus-like DNA sequences in Kaposi's sarcoma in patients with and without HIV infection. *N Engl J Med,* 332**,** 1181-5.

MORO, N., MAUCH, C. & ZIGRINO, P. 2014. Metalloproteinases in melanoma. *Eur J Cell Biol,* 93**,** 23-9.

MORTON, D. L., THOMPSON, J. F., COCHRAN, A. J., MOZZILLO, N., ELASHOFF, R., ESSNER, R., NIEWEG, O. E., ROSES, D. F., HOEKSTRA, H. J., KARAKOUSIS, C. P., REINTGEN, D. S., COVENTRY, B. J., GLASS, E. C., WANG, H. J. & GROUP, M. 2006. Sentinel-node biopsy or nodal observation in melanoma. *N Engl J Med,* 355**,** 1307-17.

MULLER, C. S. 2014. Histology of melanoma and nonmelanoma skin cancer. *Adv Exp Med Biol,* 810**,** 141-59.

MURPHY, D. A. & COURTNEIDGE, S. A. 2011. The 'ins' and 'outs' of podosomes and invadopodia: characteristics, formation and function. *Nat Rev Mol Cell Biol,* 12**,** 413-26.

MUSGROVE, E. A., CALDON, C. E., BARRACLOUGH, J., STONE, A. & SUTHERLAND, R. L. 2011. Cyclin D as a therapeutic target in cancer. *Nat Rev Cancer,* 11**,** 558-72.

MUTLU, A. D., CAVALLIN, L. E., VINCENT, L., CHIOZZINI, C., EROLES, P., DURAN, E. M., ASGARI, Z., HOOPER, A. T., LA PERLE, K. M., HILSHER, C., GAO, S. J., DITTMER, D. P., RAFII, S. & MESRI, E. A. 2007. In vivo-restricted and reversible malignancy induced by human herpesvirus-8 KSHV: a cell and animal model of virally induced Kaposi's sarcoma. *Cancer Cell,* 11**,** 245-58.

NADOR, R. G., CESARMAN, E., CHADBURN, A., DAWSON, D. B., ANSARI, M. Q., SALD, J. & KNOWLES, D. M. 1996. Primary effusion lymphoma: a distinct clinicopathologic entity associated with the Kaposi's sarcoma-associated herpes virus. *Blood,* 88**,** 645-56.

NADOR, R. G., CESARMAN, E., KNOWLES, D. M. & SAID, J. W. 1995. Herpes-like DNA sequences in a body-cavity-based lymphoma in an HIV-negative patient. *N Engl J Med,* 333**,** 943.

NAGASE, H., VISSE, R. & MURPHY, G. 2006. Structure and function of matrix metalloproteinases and TIMPs. *Cardiovasc Res,* 69**,** 562-73.

NAKAMURA, S., MURAKAMI-MORI, K., RAO, N., WEICH, H. A. & RAJEEV, B. 1997. Vascular endothelial growth factor is a potent angiogenic factor in AIDS-associated Kaposi's sarcoma-derived spindle cells. *J Immunol,* 158**,** 4992-5001.

NEIPEL, F., ALBRECHT, J. C. & FLECKENSTEIN, B. 1998. Human herpesvirus 8--the first human Rhadinovirus. *J Natl Cancer Inst Monogr***,** 73-7.

NEPKA, C., KANAKIS, D., SAMARA, M., KAPSORITAKIS, A., POTAMIANOS, S., KARANTANA, M. & KOUKOULIS, G. 2012. An unusual case of Primary Effusion Lymphoma with aberrant T-cell phenotype in a HIV-negative, HBV-positive, cirrhotic patient, and review of the literature. *Cytojournal,* 9**,** 16.

NICHOLS, L. A., ADANG, L. A. & KEDES, D. H. 2011. Rapamycin blocks production of KSHV/HHV8: insights into the anti-tumor activity of an immunosuppressant drug. *PLoS One,* 6**,** e14535.

NIESSEN, K., FU, Y., CHANG, L., HOODLESS, P. A., MCFADDEN, D. & KARSAN, A. 2008. Slug is a direct Notch target required for initiation of cardiac cushion cellularization. *J Cell Biol,* 182**,** 315-25.

NIETO, M. A. 2013. Epithelial plasticity: a common theme in embryonic and cancer cells. *Science,* 342**,** 1234850.

NIIMI, H., PARDALI, K., VANLANDEWIJCK, M., HELDIN, C. H. & MOUSTAKAS, A. 2007. Notch signaling is necessary for epithelial growth arrest by TGF-beta. *J Cell Biol,* 176**,** 695-707.

NOE, V., FINGLETON, B., JACOBS, K., CRAWFORD, H. C., VERMEULEN, S., STEELANT, W., BRUYNEEL, E., MATRISIAN, L. M. & MAREEL, M. 2001. Release of an invasion promoter E-cadherin fragment by matrilysin and stromelysin-1. *J Cell Sci,* 114**,** 111-118.

NOGUERA-TROISE, I., DALY, C., PAPADOPOULOS, N. J., COETZEE, S., BOLAND, P., GALE, N. W., LIN, H. C., YANCOPOULOS, G. D. & THURSTON, G. 2006. Blockade of Dll4 inhibits tumour growth by promoting non-productive angiogenesis. *Nature,* 444**,** 1032-7.

NOSEDA, M., FU, Y., NIESSEN, K., WONG, F., CHANG, L., MCLEAN, G. & KARSAN, A. 2006. Smooth Muscle alpha-actin is a direct target of Notch/CSL. *Circ Res,* 98**,** 1468-70.

NOY, R. & POLLARD, J. W. 2014. Tumor-associated macrophages: from mechanisms to therapy. *Immunity,* 41**,** 49-61.

O'CONNELL, M. P., MARCHBANK, K., WEBSTER, M. R., VALIGA, A. A., KAUR, A., VULTUR, A., LI, L., HERLYN, M., VILLANUEVA, J., LIU, Q., YIN, X., WIDURA, S., NELSON, J., RUIZ, N., CAMILLI, T. C., INDIG, F. E., FLAHERTY, K. T., WARGO, J. A., FREDERICK, D. T., COOPER, Z. A., NAIR, S., AMARAVADI, R. K., SCHUCHTER, L. M., KARAKOUSIS, G. C., XU, W., XU, X. & WEERARATNA, A. T. 2013. Hypoxia induces phenotypic plasticity and therapy resistance in melanoma via the tyrosine kinase receptors ROR1 and ROR2. *Cancer Discov,* 3**,** 1378-93.

OECKINGHAUS, A. & GHOSH, S. 2009. The NF-kappaB family of transcription factors and its regulation. *Cold Spring Harb Perspect Biol,* 1**,** a000034.

OJALA, P. M., TIAINEN, M., SALVEN, P., VEIKKOLA, T., CASTANOS-VELEZ, E., SARID, R., BIBERFELD, P. & MAKELA, T. P. 1999. Kaposi's sarcoma-associated herpesvirus-encoded v-cyclin triggers apoptosis in cells with high levels of cyclin-dependent kinase 6. *Cancer Res,* 59**,** 4984-9.

OJALA, P. M., YAMAMOTO, K., CASTANOS-VELEZ, E., BIBERFELD, P., KORSMEYER, S. J. & MAKELA, T. P. 2000. The apoptotic v-cyclin-CDK6 complex phosphorylates and inactivates Bcl-2. *Nat Cell Biol,* 2**,** 819-25.

OLUMI, A. F., GROSSFELD, G. D., HAYWARD, S. W., CARROLL, P. R., TLSTY, T. D. & CUNHA, G. R. 1999. Carcinoma-associated fibroblasts direct tumor progression of initiated human prostatic epithelium. *Cancer Res,* 59**,** 5002-11.

ORIMO, A., GUPTA, P. B., SGROI, D. C., ARENZANA-SEISDEDOS, F., DELAUNAY, T., NAEEM, R., CAREY, V. J., RICHARDSON, A. L. & WEINBERG, R. A. 2005. Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. *Cell,* 121**,** 335-48.

ORY, D. S., NEUGEBOREN, B. A. & MULLIGAN, R. C. 1996. A stable human-derived packaging cell line for production of high titer retrovirus/vesicular stomatitis virus G pseudotypes. *Proc Natl Acad Sci U S A,* 93**,** 11400-6.

OSIPO, C., GOLDE, T. E., OSBORNE, B. A. & MIELE, L. A. 2008. Off the beaten pathway: the complex cross talk between Notch and NF-kappaB. *Lab Invest,* 88**,** 11-7.

OVASKA, K., LAAKSO, M., HAAPA-PAANANEN, S., LOUHIMO, R., CHEN, P., AITTOMAKI, V., VALO, E., NUNEZ-FONTARNAU, J., RANTANEN, V., KARINEN, S., NOUSIAINEN, K., LAHESMAA-KORPINEN, A. M., MIETTINEN, M., SAARINEN, L., KOHONEN, P., WU, J., WESTERMARCK, J. & HAUTANIEMI, S. 2010. Large-scale data integration framework provides a comprehensive view on glioblastoma multiforme. *Genome Med,* 2**,** 65.

OVERALL, C. M. & BLOBEL, C. P. 2007. In search of partners: linking extracellular proteases to substrates. *Nat Rev Mol Cell Biol,* 8**,** 245-57.

PATENAUDE, A., PARKER, J. & KARSAN, A. 2010. Involvement of endothelial progenitor cells in tumor vascularization. *Microvasc Res,* 79**,** 217-23.

PEAR, W. S., ASTER, J. C., SCOTT, M. L., HASSERJIAN, R. P., SOFFER, B., SKLAR, J. & BALTIMORE, D. 1996. Exclusive development of T cell neoplasms in mice transplanted with bone marrow expressing activated Notch alleles. *J Exp Med,* 183**,** 2283-91.

PEINADO, H., PORTILLO, F. & CANO, A. 2004. Transcriptional regulation of cadherins during development and carcinogenesis. *Int J Dev Biol,* 48**,** 365-75.

PEPPICELLI, S., BIANCHINI, F. & CALORINI, L. 2014. Inflammatory cytokines induce vascular endothelial growth factor-C expression in melanoma-associated macrophages and stimulate melanoma lymph node metastasis. *Oncol Lett,* 8**,** 1133-1138.

PEPPICELLI, S., BIANCHINI, F., CONTENA, C., TOMBACCINI, D. & CALORINI, L. 2013. Acidic pH via NF-kappaB favours VEGF-C expression in human melanoma cells. *Clin Exp Metastasis,* 30**,** 957-67.

PETROVA, T. V., MAKINEN, T., MAKELA, T. P., SAARELA, J., VIRTANEN, I., FERRELL, R. E., FINEGOLD, D. N., KERJASCHKI, D., YLA-HERTTUALA, S. & ALITALO, K. 2002. Lymphatic endothelial reprogramming of vascular endothelial cells by the Prox-1 homeobox transcription factor. *EMBO J,* 21**,** 4593-9.

PINNIX, C. C. & HERLYN, M. 2007. The many faces of Notch signaling in skin-derived cells. *Pigment Cell Res,* 20**,** 458-65.

POTENTA, S., ZEISBERG, E. & KALLURI, R. 2008. The role of endothelial-to-mesenchymal transition in cancer progression. *Br J Cancer,* 99**,** 1375-9.

QIAN, L. W., GREENE, W., YE, F. & GAO, S. J. 2008. Kaposi's sarcoma-associated herpesvirus disrupts adherens junctions and increases endothelial permeability by inducing degradation of VE-cadherin. *J Virol,* 82**,** 11902-12.

QIAN, L. W., XIE, J., YE, F. & GAO, S. J. 2007. Kaposi's sarcoma-associated herpesvirus infection promotes invasion of primary human umbilical vein endothelial cells by inducing matrix metalloproteinases. *J Virol,* 81**,** 7001-10.

QUAIL, D. F. & JOYCE, J. A. 2013. Microenvironmental regulation of tumor progression and metastasis. *Nat Med,* 19**,** 1423-37.

QUANTE, M., TU, S. P., TOMITA, H., GONDA, T., WANG, S. S., TAKASHI, S., BAIK, G. H., SHIBATA, W., DIPRETE, B., BETZ, K. S., FRIEDMAN, R., VARRO, A., TYCKO, B. & WANG, T. C. 2011. Bone marrow-derived myofibroblasts contribute to the mesenchymal stem cell niche and promote tumor growth. *Cancer Cell,* 19**,** 257-72.

RADISKY, D. C., KENNY, P. A. & BISSELL, M. J. 2007. Fibrosis and cancer: do myofibroblasts come also from epithelial cells via EMT? *J Cell Biochem,* 101**,** 830-9.

RADKOV, S. A., KELLAM, P. & BOSHOFF, C. 2000. The latent nuclear antigen of Kaposi sarcoma-associated herpesvirus targets the retinoblastoma-E2F pathway and with the oncogene Hras transforms primary rat cells. *Nat Med,* 6**,** 1121-7.

RADU, O. & PANTANOWITZ, L. 2013. Kaposi sarcoma. *Arch Pathol Lab Med,* 137**,** 289-94.

RAFFAGHELLO, L., COCCO, C., CORRIAS, M. V., AIROLDI, I. & PISTOIA, V. 2009. Chemokines in neuroectodermal tumour progression and metastasis. *Semin Cancer Biol,* 19**,** 97-102.

RAGGO, C., RUHL, R., MCALLISTER, S., KOON, H., DEZUBE, B. J., FRUH, K. & MOSES, A. V. 2005. Novel cellular genes essential for transformation of endothelial cells by Kaposi's sarcoma-associated herpesvirus. *Cancer Res,* 65**,** 5084-95.

RANGARAJAN, A., TALORA, C., OKUYAMA, R., NICOLAS, M., MAMMUCARI, C., OH, H., ASTER, J. C., KRISHNA, S., METZGER, D., CHAMBON, P., MIELE, L., AGUET, M., RADTKE, F. & DOTTO, G. P. 2001. Notch signaling is a direct determinant of keratinocyte growth arrest and entry into differentiation. *EMBO J,* 20**,** 3427-36.

RASTRELLI, M., TROPEA, S., ROSSI, C. R. & ALAIBAC, M. 2014. Melanoma: Epidemiology, Risk Factors, Pathogenesis, Diagnosis and Classification. *In Vivo,* 28**,** 1005-1011.

REDONDO-MUNOZ, J., UGARTE-BERZAL, E., GARCIA-MARCO, J. A., DEL CERRO, M. H., VAN DEN STEEN, P. E., OPDENAKKER, G., TEROL, M. J. & GARCIA-PARDO, A. 2008. Alpha4beta1 integrin and 190-kDa CD44v constitute a cell surface docking complex for gelatinase B/MMP-9 in chronic leukemic but not in normal B cells. *Blood,* 112**,** 169-78.

REYMOND, N., D'AGUA, B. B. & RIDLEY, A. J. 2013. Crossing the endothelial barrier during metastasis. *Nat Rev Cancer,* 13**,** 858-70.

RIDGWAY, J., ZHANG, G., WU, Y., STAWICKI, S., LIANG, W. C., CHANTHERY, Y., KOWALSKI, J., WATTS, R. J., CALLAHAN, C., KASMAN, I., SINGH, M., CHIEN, M., TAN, C., HONGO, J. A., DE SAUVAGE, F., PLOWMAN, G. & YAN, M. 2006. Inhibition of Dll4 signalling inhibits tumour growth by deregulating angiogenesis. *Nature,* 444**,** 1083-7.

ROY, D., SIN, S. H., LUCAS, A., VENKATARAMANAN, R., WANG, L., EASON, A., CHAVAKULA, V., HILTON, I. B., TAMBURRO, K. M., DAMANIA, B. & DITTMER, D. P. 2013. mTOR inhibitors block Kaposi sarcoma growth by inhibiting essential autocrine growth factors and tumor angiogenesis. *Cancer Res,* 73**,** 2235-46.

SAHLGREN, C., GUSTAFSSON, M. V., JIN, S., POELLINGER, L. & LENDAHL, U. 2008. Notch signaling mediates hypoxia-induced tumor cell migration and invasion. *Proc Natl Acad Sci U S A,* 105**,** 6392-7.

SAID, J. W., SHINTAKU, I. P., ASOU, H., DEVOS, S., BAKER, J., HANSON, G., CESARMAN, E., NADOR, R. & KOEFFLER, H. P. 1999. Herpesvirus 8 inclusions in primary effusion lymphoma: report of a unique case with T-cell phenotype. *Arch Pathol Lab Med,* 123**,** 257-60.

SAITO, A. 2013. EMT and EndMT: regulated in similar ways? *J Biochem,* 153**,** 493-5.

SAKAKIBARA, S., PISE-MASISON, C. A., BRADY, J. N. & TOSATO, G. 2009. Gene regulation and functional alterations induced by Kaposi's sarcoma-associated herpesvirus-encoded ORFK13/vFLIP in endothelial cells. *J Virol,* 83**,** 2140-53.

SANTAMARIA, D., BARRIERE, C., CERQUEIRA, A., HUNT, S., TARDY, C., NEWTON, K., CACERES, J. F., DUBUS, P., MALUMBRES, M. & BARBACID, M. 2007. Cdk1 is sufficient to drive the mammalian cell cycle. *Nature,* 448**,** 811-5.

SANTAMARIA, D. & ORTEGA, S. 2006. Cyclins and CDKS in development and cancer: lessons from genetically modified mice. *Front Biosci,* 11**,** 1164-88.

SAREK, G., JARVILUOMA, A. & OJALA, P. M. 2006. KSHV viral cyclin inactivates p27KIP1 through Ser10 and Thr187 phosphorylation in proliferating primary effusion lymphomas. *Blood,* 107**,** 725-32.

SAREK, G., KURKI, S., ENBACK, J., IOTZOVA, G., HAAS, J., LAAKKONEN, P., LAIHO, M. & OJALA, P. M. 2007. Reactivation of the p53 pathway as a treatment modality for KSHV-induced lymphomas. *J Clin Invest,* 117**,** 1019-28.

SAWAI, C. M., FREUND, J., OH, P., NDIAYE-LOBRY, D., BRETZ, J. C., STRIKOUDIS, A., GENESCA, L., TRIMARCHI, T., KELLIHER, M. A., CLARK, M., SOULIER, J., CHEN-KIANG, S. & AIFANTIS, I. 2012. Therapeutic targeting of the cyclin D3:CDK4/6 complex in T cell leukemia. *Cancer Cell,* 22**,** 452-65.

SCHMID, J. A. & BIRBACH, A. 2008. IkappaB kinase beta (IKKbeta/IKK2/IKBKB)--a key molecule in signaling to the transcription factor NF-kappaB. *Cytokine Growth Factor Rev,* 19**,** 157-65.

SCHMITT, C. A., FRIDMAN, J. S., YANG, M., BARANOV, E., HOFFMAN, R. M. & LOWE, S. W. 2002. Dissecting p53 tumor suppressor functions in vivo. *Cancer Cell,* 1**,** 289-98.

SCHULZE-OSTHOFF, K., FERRARI, D., RIEHEMANN, K. & WESSELBORG, S. 1997. Regulation of NF-kappa B activation by MAP kinase cascades. *Immunobiology,* 198**,** 35-49.

SEFTOR, R. E., HESS, A. R., SEFTOR, E. A., KIRSCHMANN, D. A., HARDY, K. M., MARGARYAN, N. V. & HENDRIX, M. J. 2012. Tumor cell vasculogenic mimicry: from controversy to therapeutic promise. *Am J Pathol,* 181**,** 1115-25.

SEMI, K., MATSUDA, Y., OHNISHI, K. & YAMADA, Y. 2013. Cellular reprogramming and cancer development. *Int J Cancer,* 132**,** 1240-8.

SEVENICH, L. & JOYCE, J. A. 2014. Pericellular proteolysis in cancer. *Genes Dev,* 28**,** 2331-2347.

SHIELDS, J. D., FLEURY, M. E., YONG, C., TOMEI, A. A., RANDOLPH, G. J. & SWARTZ, M. A. 2007. Autologous chemotaxis as a mechanism of tumor cell homing to lymphatics via interstitial flow and autocrine CCR7 signaling. *Cancer Cell,* 11**,** 526-38.

SICINSKA, E., AIFANTIS, I., LE CAM, L., SWAT, W., BOROWSKI, C., YU, Q., FERRANDO, A. A., LEVIN, S. D., GENG, Y., VON BOEHMER, H. & SICINSKI, P. 2003. Requirement for cyclin D3 in lymphocyte development and T cell leukemias. *Cancer Cell,* 4**,** 451-61.

SIN, S. H. & DITTMER, D. P. 2013. Viral latency locus augments B-cell response in vivo to induce chronic marginal zone enlargement, plasma cell hyperplasia, and lymphoma. *Blood,* 121**,** 2952-63.

SKOBE, M., HAWIGHORST, T., JACKSON, D. G., PREVO, R., JANES, L., VELASCO, P., RICCARDI, L., ALITALO, K., CLAFFEY, K. & DETMAR, M. 2001. Induction of tumor lymphangiogenesis by VEGF-C promotes breast cancer metastasis. *Nat Med,* 7**,** 192-8.

SLEEMAN, J., SCHMID, A. & THIELE, W. 2009. Tumor lymphatics. *Semin Cancer Biol,* 19**,** 285-97.

SLEEMAN, J. P. 2012. The metastatic niche and stromal progression. *Cancer Metastasis Rev,* 31**,** 429-40.

SLEEMAN, J. P., NAZARENKO, I. & THIELE, W. 2011. Do all roads lead to Rome? Routes to metastasis development. *Int J Cancer,* 128**,** 2511-26.

SLEEMAN, J. P. & THIELE, W. 2009. Tumor metastasis and the lymphatic vasculature. *Int J Cancer,* 125**,** 2747-56.

SODHI, A., CHAISUPARAT, R., HU, J., RAMSDELL, A. K., MANNING, B. D., SAUSVILLE, E. A., SAWAI, E. T., MOLINOLO, A., GUTKIND, J. S. & MONTANER, S. 2006. The TSC2/mTOR pathway drives endothelial cell transformation induced by the Kaposi's sarcoma-associated herpesvirus G protein-coupled receptor. *Cancer Cell,* 10**,** 133-43.

SODHI, A., MONTANER, S. & GUTKIND, J. S. 2004a. Viral hijacking of G-protein-coupled-receptor signalling networks. *Nat Rev Mol Cell Biol,* 5**,** 998-1012.

SODHI, A., MONTANER, S., PATEL, V., GOMEZ-ROMAN, J. J., LI, Y., SAUSVILLE, E. A., SAWAI, E. T. & GUTKIND, J. S. 2004b. Akt plays a central role in sarcomagenesis induced by Kaposi's sarcoma herpesvirus-encoded G protein-coupled receptor. *Proc Natl Acad Sci U S A,* 101**,** 4821-6.

SODHI, A., MONTANER, S., PATEL, V., ZOHAR, M., BAIS, C., MESRI, E. A. & GUTKIND, J. S. 2000. The Kaposi's sarcoma-associated herpes virus G protein-coupled receptor up-regulates vascular endothelial growth factor expression and secretion through mitogen-activated protein kinase and p38 pathways acting on hypoxia-inducible factor 1alpha. *Cancer Res,* 60**,** 4873-80.

SOULIER, J., GROLLET, L., OKSENHENDLER, E., CACOUB, P., CAZALS-HATEM, D., BABINET, P., D'AGAY, M. F., CLAUVEL, J. P., RAPHAEL, M., DEGOS, L. & ET AL. 1995. Kaposi's sarcoma-associated herpesvirus-like DNA sequences in multicentric Castleman's disease. *Blood,* 86**,** 1276-80.

SPAETH, E. L., DEMBINSKI, J. L., SASSER, A. K., WATSON, K., KLOPP, A., HALL, B., ANDREEFF, M. & MARINI, F. 2009. Mesenchymal stem cell transition to tumor-associated fibroblasts contributes to fibrovascular network expansion and tumor progression. *PLoS One,* 4**,** e4992.

SRIVASTAVA, K., HU, J., KORN, C., SAVANT, S., TEICHERT, M., KAPEL, S. S., JUGOLD, M., BESEMFELDER, E., THOMAS, M., PASPARAKIS, M. & AUGUSTIN, H. G. 2014. Postsurgical adjuvant tumor therapy by combining anti-angiopoietin-2 and metronomic chemotherapy limits metastatic growth. *Cancer Cell,* 26**,** 880-95.

STACKER, S. A., CAESAR, C., BALDWIN, M. E., THORNTON, G. E., WILLIAMS, R. A., PREVO, R., JACKSON, D. G., NISHIKAWA, S., KUBO, H. & ACHEN, M. G. 2001. VEGF-D promotes the metastatic spread of tumor cells via the lymphatics. *Nat Med,* 7**,** 186-91.

STERNBACH, G. & VARON, J. 1995. Moritz Kaposi: idiopathic pigmented sarcoma of the skin. *J Emerg Med,* 13**,** 671-4.

STERNLICHT, M. D. & WERB, Z. 2001. How matrix metalloproteinases regulate cell behavior. *Annu Rev Cell Dev Biol,* 17**,** 463-516.

STEWART, K. S., ZHOU, Z., ZWEIDLER-MCKAY, P. & KLEINERMAN, E. S. 2011. Delta-like ligand 4-Notch signaling regulates bone marrow-derived pericyte/vascular smooth muscle cell formation. *Blood,* 117**,** 719-26.

STURZL, M., BRANDSTETTER, H., ZIETZ, C., EISENBURG, B., RAIVICH, G., GEARING, D. P., BROCKMEYER, N. H. & HOFSCHNEIDER, P. H. 1995. Identification of interleukin-1 and platelet-derived growth factor-B as major mitogens for the spindle cells of Kaposi's sarcoma: a combined in vitro and in vivo analysis. *Oncogene,* 10**,** 2007-16.

SUETSUGU, A., OSAWA, Y., NAGAKI, M., SAJI, S., MORIWAKI, H., BOUVET, M. & HOFFMAN, R. M. 2011. Imaging the recruitment of cancer-associated fibroblasts by liver-metastatic colon cancer. *J Cell Biochem,* 112**,** 949-53.

SUFFERT, G., MALTERER, G., HAUSSER, J., VIILIAINEN, J., FENDER, A., CONTRANT, M., IVACEVIC, T., BENES, V., GROS, F., VOINNET, O., ZAVOLAN, M., OJALA, P. M., HAAS, J. G. & PFEFFER, S. 2011. Kaposi's sarcoma herpesvirus microRNAs target caspase 3 and regulate apoptosis. *PLoS Pathog,* 7**,** e1002405.

SUGIMOTO, H., MUNDEL, T. M., KIERAN, M. W. & KALLURI, R. 2006. Identification of fibroblast heterogeneity in the tumor microenvironment. *Cancer Biol Ther,* 5**,** 1640-6.

SUN, R., LIN, S. F., STASKUS, K., GRADOVILLE, L., GROGAN, E., HAASE, A. & MILLER, G. 1999. Kinetics of Kaposi's sarcoma-associated herpesvirus gene expression. *J Virol,* 73**,** 2232-42.

SUN, S. C., CHANG, J. H. & JIN, J. 2013. Regulation of nuclear factor-kappaB in autoimmunity. *Trends Immunol,* 34**,** 282-9.

SWANTON, C., MANN, D. J., FLECKENSTEIN, B., NEIPEL, F., PETERS, G. & JONES, N. 1997. Herpes viral cyclin/Cdk6 complexes evade inhibition by CDK inhibitor proteins. *Nature,* 390**,** 184-7.

TAKAHASHI, K. & YAMANAKA, S. 2006. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell,* 126**,** 663-76.

TALBOT, S. J., WEISS, R. A., KELLAM, P. & BOSHOFF, C. 1999. Transcriptional analysis of human herpesvirus-8 open reading frames 71, 72, 73, K14, and 74 in a primary effusion lymphoma cell line. *Virology,* 257**,** 84-94.

TANIGAKI, K., HAN, H., YAMAMOTO, N., TASHIRO, K., IKEGAWA, M., KURODA, K., SUZUKI, A., NAKANO, T. & HONJO, T. 2002. Notch-RBP-J signaling is involved in cell fate determination of marginal zone B cells. *Nat Immunol,* 3**,** 443-50.

TANIGAKI, K., TSUJI, M., YAMAMOTO, N., HAN, H., TSUKADA, J., INOUE, H., KUBO, M. & HONJO, T. 2004. Regulation of alphabeta/gammadelta T cell lineage commitment and peripheral T cell responses by Notch/RBP-J signaling. *Immunity,* 20**,** 611-22.

THIERY, J. P. 2002. Epithelial-mesenchymal transitions in tumour progression. *Nat Rev Cancer,* 2**,** 442-54.

THURAU, M., MARQUARDT, G., GONIN-LAURENT, N., WEINLANDER, K., NASCHBERGER, E., JOCHMANN, R., ALKHARSAH, K. R., SCHULZ, T. F., THOME, M., NEIPEL, F. & STURZL, M. 2009. Viral inhibitor of apoptosis vFLIP/K13 protects endothelial cells against superoxide-induced cell death. *J Virol,* 83**,** 598-611.

TOPCZEWSKA, J. M., POSTOVIT, L. M., MARGARYAN, N. V., SAM, A., HESS, A. R., WHEATON, W. W., NICKOLOFF, B. J., TOPCZEWSKI, J. & HENDRIX, M. J. 2006. Embryonic and tumorigenic pathways converge via Nodal signaling: role in melanoma aggressiveness. *Nat Med,* 12**,** 925-32.

TOTONCHY, J. E., CLEPPER, L., PHILLIPS, K. G., MCCARTY, O. J. & MOSES, A. V. 2014. CXCR7 expression disrupts endothelial cell homeostasis and causes ligand-dependent invasion. *Cell Adh Migr,* 8**,** 165-76.

VANDEWALLE, C., COMIJN, J., DE CRAENE, B., VERMASSEN, P., BRUYNEEL, E., ANDERSEN, H., TULCHINSKY, E., VAN ROY, F. & BERX, G. 2005. SIP1/ZEB2 induces EMT by repressing genes of different epithelial cell-cell junctions. *Nucleic Acids Res,* 33**,** 6566-78.

VERMA, S. C., CHOUDHURI, T., KAUL, R. & ROBERTSON, E. S. 2006. Latency-associated nuclear antigen (LANA) of Kaposi's sarcoma-associated herpesvirus interacts with origin recognition complexes at the LANA binding sequence within the terminal repeats. *J Virol,* 80**,** 2243-56.

VERSCHUREN, E. W., HODGSON, J. G., GRAY, J. W., KOGAN, S., JONES, N. & EVAN, G. I. 2004a. The role of p53 in suppression of KSHV cyclin-induced lymphomagenesis. *Cancer Res,* 64**,** 581-9.

VERSCHUREN, E. W., JONES, N. & EVAN, G. I. 2004b. The cell cycle and how it is steered by Kaposi's sarcoma-associated herpesvirus cyclin. *J Gen Virol,* 85**,** 1347-61.

VERSCHUREN, E. W., KLEFSTROM, J., EVAN, G. I. & JONES, N. 2002. The oncogenic potential of Kaposi's sarcoma-associated herpesvirus cyclin is exposed by p53 loss in vitro and in vivo. *Cancer Cell,* 2**,** 229-41.

VIEIRA, J. & O'HEARN, P. M. 2004. Use of the red fluorescent protein as a marker of Kaposi's sarcoma-associated herpesvirus lytic gene expression. *Virology,* 325**,** 225-40.

VILLANUEVA, J. & HERLYN, M. 2008. Melanoma and the tumor microenvironment. *Curr Oncol Rep,* 10**,** 439-46.

VULTUR, A., VILLANUEVA, J., KREPLER, C., RAJAN, G., CHEN, Q., XIAO, M., LI, L., GIMOTTY, P. A., WILSON, M., HAYDEN, J., KEENEY, F., NATHANSON, K. L. & HERLYN, M. 2014. MEK inhibition affects STAT3 signaling and invasion in human melanoma cell lines. *Oncogene,* 33**,** 1850-61.

WANG, H. W., TROTTER, M. W., LAGOS, D., BOURBOULIA, D., HENDERSON, S., MAKINEN, T., ELLIMAN, S., FLANAGAN, A. M., ALITALO, K. & BOSHOFF, C. 2004. Kaposi sarcoma herpesvirus-induced cellular reprogramming contributes to the lymphatic endothelial gene expression in Kaposi sarcoma. *Nat Genet,* 36**,** 687-93.

WANG, L. & DAMANIA, B. 2008. Kaposi's sarcoma-associated herpesvirus confers a survival advantage to endothelial cells. *Cancer Res,* 68**,** 4640-8.

WANG, L. X., KANG, G., KUMAR, P., LU, W., LI, Y., ZHOU, Y., LI, Q. & WOOD, C. 2014. Humanized-BLT mouse model of Kaposi's sarcoma-associated herpesvirus infection. *Proc Natl Acad Sci U S A,* 111**,** 3146-51.

WANG, Y., LIU, Y., MALEK, S. N., ZHENG, P. & LIU, Y. 2011. Targeting HIF1alpha eliminates cancer stem cells in hematological malignancies. *Cell Stem Cell,* 8**,** 399-411.

WANG, Y., NAKAYAMA, M., PITULESCU, M. E., SCHMIDT, T. S., BOCHENEK, M. L., SAKAKIBARA, A., ADAMS, S., DAVY, A., DEUTSCH, U., LUTHI, U., BARBERIS, A., BENJAMIN, L. E., MAKINEN, T., NOBES, C. D. & ADAMS, R. H. 2010. Ephrin-B2 controls VEGF-induced angiogenesis and lymphangiogenesis. *Nature,* 465**,** 483-6.

WEBSTER, G. A. & PERKINS, N. D. 1999. Transcriptional cross talk between NF-kappaB and p53. *Mol Cell Biol,* 19**,** 3485-95.

WEICH, H. A., SALAHUDDIN, S. Z., GILL, P., NAKAMURA, S., GALLO, R. C. & FOLKMANN, J. 1991. AIDS-associated Kaposi's sarcoma-derived cells in long-term culture express and synthesize smooth muscle alpha-actin. *Am J Pathol,* 139**,** 1251-8.

WELCH-REARDON, K. M., EHSAN, S. M., WANG, K., WU, N., NEWMAN, A. C., ROMERO-LOPEZ, M., FONG, A. H., GEORGE, S. C., EDWARDS, R. A. & HUGHES, C. C. 2014a. Angiogenic sprouting is regulated by endothelial cell expression of Slug. *J Cell Sci,* 127**,** 2017-28.

WELCH-REARDON, K. M., WU, N. & HUGHES, C. C. 2014b. A Role for Partial Endothelial-Mesenchymal Transitions in Angiogenesis? *Arterioscler Thromb Vasc Biol*.

WENG, A. P., FERRANDO, A. A., LEE, W., MORRIS, J. P. T., SILVERMAN, L. B., SANCHEZ-IRIZARRY, C., BLACKLOW, S. C., LOOK, A. T. & ASTER, J. C. 2004. Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. *Science,* 306**,** 269-71.

WHITEMAN, D. C., WHITEMAN, C. A. & GREEN, A. C. 2001. Childhood sun exposure as a risk factor for melanoma: a systematic review of epidemiologic studies. *Cancer Causes Control,* 12**,** 69-82.

WOLF, K., TE LINDERT, M., KRAUSE, M., ALEXANDER, S., TE RIET, J., WILLIS, A. L., HOFFMAN, R. M., FIGDOR, C. G., WEISS, S. J. & FRIEDL, P. 2013. Physical limits of cell migration: control by ECM space and nuclear deformation and tuning by proteolysis and traction force. *J Cell Biol,* 201**,** 1069-84.

XIE, T. X., XIA, Z., ZHANG, N., GONG, W. & HUANG, S. 2010. Constitutive NF-kappaB activity regulates the expression of VEGF and IL-8 and tumor angiogenesis of human glioblastoma. *Oncol Rep,* 23**,** 725-32.

YANAI, Y., FURUHATA, T., KIMURA, Y., YAMAGUCHI, K., YASOSHIMA, T., MITAKA, T., MOCHIZUKI, Y. & HIRATA, K. 2001. Vascular endothelial growth factor C promotes human gastric carcinoma lymph node metastasis in mice. *J Exp Clin Cancer Res,* 20**,** 419-28.

YANG, J., MANI, S. A., DONAHER, J. L., RAMASWAMY, S., ITZYKSON, R. A., COME, C., SAVAGNER, P., GITELMAN, I., RICHARDSON, A. & WEINBERG, R. A. 2004. Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. *Cell,* 117**,** 927-39.

YANG, J., SPLITTGERBER, R., YULL, F. E., KANTROW, S., AYERS, G. D., KARIN, M. & RICHMOND, A. 2010. Conditional ablation of Ikkb inhibits melanoma tumor development in mice. *J Clin Invest,* 120**,** 2563-74.

YE, F., ZHOU, F., BEDOLLA, R. G., JONES, T., LEI, X., KANG, T., GUADALUPE, M. & GAO, S. J. 2011. Reactive oxygen species hydrogen peroxide mediates Kaposi's sarcoma-associated herpesvirus reactivation from latency. *PLoS Pathog,* 7**,** e1002054.

YEO, E. J., CASSETTA, L., QIAN, B. Z., LEWKOWICH, I., LI, J. F., STEFATER, J. A., 3RD, SMITH, A. N., WIECHMANN, L. S., WANG, Y., POLLARD, J. W. & LANG, R. A. 2014. Myeloid WNT7b mediates the angiogenic switch and metastasis in breast cancer. *Cancer Res,* 74**,** 2962-73.

YOO, S. M., JANG, J., YOO, C. & LEE, M. S. 2014. Kaposi's sarcoma-associated herpesvirus infection of human bone-marrow-derived mesenchymal stem cells and their angiogenic potential. *Arch Virol,* 159**,** 2377-86.

YU, Q., GENG, Y. & SICINSKI, P. 2001. Specific protection against breast cancers by cyclin D1 ablation. *Nature,* 411**,** 1017-21.

YU, Q., SICINSKA, E., GENG, Y., AHNSTROM, M., ZAGOZDZON, A., KONG, Y., GARDNER, H., KIYOKAWA, H., HARRIS, L. N., STAL, O. & SICINSKI, P. 2006. Requirement for CDK4 kinase function in breast cancer. *Cancer Cell,* 9**,** 23-32.

YU, Y., BLACK, J. B., GOLDSMITH, C. S., BROWNING, P. J., BHALLA, K. & OFFERMANN, M. K. 1999. Induction of human herpesvirus-8 DNA replication and transcription by butyrate and TPA in BCBL-1 cells. *J Gen Virol,* 80 (Pt 1)**,** 83-90.

ZAVADIL, J., CERMAK, L., SOTO-NIEVES, N. & BOTTINGER, E. P. 2004. Integration of TGF-beta/Smad and Jagged1/Notch signalling in epithelial-to-mesenchymal transition. *EMBO J,* 23**,** 1155-65.

ZEISBERG, E. M., POTENTA, S., XIE, L., ZEISBERG, M. & KALLURI, R. 2007a. Discovery of endothelial to mesenchymal transition as a source for carcinoma-associated fibroblasts. *Cancer Res,* 67**,** 10123-8.

ZEISBERG, E. M., TARNAVSKI, O., ZEISBERG, M., DORFMAN, A. L., MCMULLEN, J. R., GUSTAFSSON, E., CHANDRAKER, A., YUAN, X., PU, W. T., ROBERTS, A. B., NEILSON, E. G., SAYEGH, M. H., IZUMO, S. & KALLURI, R. 2007b. Endothelial-to-mesenchymal transition contributes to cardiac fibrosis. *Nat Med,* 13**,** 952-61.

ZHANG, P., GOODRICH, C., FU, C. & DONG, C. 2014. Melanoma upregulates ICAM-1 expression on endothelial cells through engagement of tumor CD44 with endothelial E-selectin and activation of a PKCalpha-p38-SP-1 pathway. *FASEB J*.

ZHANG, X. H., JIN, X., MALLADI, S., ZOU, Y., WEN, Y. H., BROGI, E., SMID, M., FOEKENS, J. A. & MASSAGUE, J. 2013. Selection of bone metastasis seeds by mesenchymal signals in the primary tumor stroma. *Cell,* 154**,** 1060-73.

ZHENG, W., ASPELUND, A. & ALITALO, K. 2014a. Lymphangiogenic factors, mechanisms, and applications. *J Clin Invest,* 124**,** 878-87.

ZHENG, W., NURMI, H., APPAK, S., SABINE, A., BOVAY, E., KORHONEN, E. A., ORSENIGO, F., LOHELA, M., D'AMICO, G., HOLOPAINEN, T., LEOW, C. C., DEJANA, E., PETROVA, T. V., AUGUSTIN, H. G. & ALITALO, K. 2014b. Angiopoietin 2 regulates the transformation and integrity of lymphatic endothelial cell junctions. *Genes Dev,* 28**,** 1592-603.

ZHENG, W., TAMMELA, T., YAMAMOTO, M., ANISIMOV, A., HOLOPAINEN, T., KAIJALAINEN, S., KARPANEN, T., LEHTI, K., YLA-HERTTUALA, S. & ALITALO, K. 2011. Notch restricts lymphatic vessel sprouting induced by vascular endothelial growth factor. *Blood,* 118**,** 1154-62.

ZHI, H., ZAHOOR, M. A., SHUDOFSKY, A. M. & GIAM, C. Z. 2014. KSHV vCyclin counters the senescence/G1 arrest response triggered by NF-kappaB hyperactivation. *Oncogene*.