Charles University

Faculty of Science

Study program: Special Chemical and Biological Programmes

Branch of study: Molecular Biology and Biochemistry of Organisms

Michaela Remišová

The role of galectins in cancer cell invasiveness

Úloha galektinů v invazivitě nádorových buněk

Bachelor´s thesis

Supervisor: Doc. RNDr. Jan Brábek, Ph.D.

Prague, 2018

Poděkovaní patří mému školiteli Doc. RNDr. Janu Brábkovi, Ph.D. za pomoc, odborné konzultace a trpělivost.

Prohlášení:

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, 23.4.2018

Michaela Remišová

……

Abstract

Galectins are family of β-galactosidase binding proteins that serve many functions in all kind of mammalian cells. In the past years galectins, namely galectin-1 and galectin-3, have been revealed to play a major role in various cancer processes including cancer cell invasiveness, a process indispensable for the formation of metastasis. Both extracellular and intracellular forms of galectins modify the process of invasiveness in various ways, through interacting with different components of the cell or of the cell signalling pathways. The aim of this bachelor's thesis is to summarize mechanisms by which galectins promote cancer cell invasiveness.

Keywords: galectins, galectin-1, galectin-3, invasiveness, cancer, metastasis

Abstrakt

Galektiny jsou skupinou proteinů vázajících β-galaktozidázu, které mají mnoho funkcí ve všech druzích savčích buněk. V uplynulých letech se ukázalo, že galektiny, jmenovitě galektin-1 a galektin-3, mají důležitou roli v procesech nádorové progrese, včetně invazivity nádorových buněk, procesu nezbytného pro tvorbu metastáz. Jak extracelulární, tak intracelulární formy galektinů modifikují proces invazivity různými způsoby, a to interakcí s různými komponentami buňky nebo buněčných signalizačních drah. Cílem této bakalářské práce je shrnout mechanismy, kterými galektiny zvyšují invazivitu nádorových buněk.

Klíčová slova: galektiny, galektin-1, galektin-3, invazivita, nádorová onemocnění, metastázy

Abbreviations

ECM	extracellular matrix
CRD	carbohydrate recognition domain
ABC	ATP binding cassette
PCDH24	Protocadherin-24
Ras	Retrovirus associated DNA sequence
Gemin4	Gem nuclear organelle associated protein 4
MAC-2BP	Mac-2 binding protein
Alix	ALG-2-interacting protein X
EGFR	Epidermal growth factor receptor
Bcl2	B-cell lymphoma 2 protein
Chrp	Cysteine- and histidine-rich cytoplasmic protein
APC	Adenomatous polyposis coli
GSK β	Glycogen synthase kinase 3 β
TCF	T-cell factor
Lef1	Lymphoid enhancer factor-1
MMP	Matrix metalloproteinase
FAK	Focal adhesion kinase
MAPK	Mitogen-activated protein kinase
NF-κB	Nuclear factor-κB
JNK1/2/3	Jun amino-terminal kinases
Erk	Extracellular signal-regulated kinase
GAP	GTPase activating protein
GEF	Guanine nucleotide exchange factor
PAR	Protease activated receptor
RhoA	Ras homolog gene family member A

Cdc42	Cell division control protein 42 homolog
MLCK	Myosin light chain kinase
MYPT1	Myosin phosphatase
ROCK	Rho-associated protein kinase
Cav-1	Caveolin-1
Mgat5	Mannosyl (α-1,6)-glycoprotein β-1,6-*N*-acetyl-glucosamintransferase
PTRF	Polymerase I transcription release factor
Akt	Protein kinase B
PI3K	Phosphoinositide 3-kinase
EMT	Epithelial-mesenchymal transition
PSC	Pancreatic stellate cells
SMO	Smoothened
PTCH	Patched
uPAR	Urokinase-type plasminogen activator receptor
pro-uPA	Pro-urokinase-type plasminogen activator
uPA	Urokinase-type plasminogen activator
TF	Thomsen-Friedenreich antigen
MCP	Modified pectin
IκBα	B-cells inhibitor α
IKK	NF-κB kinase complex
NIK	Process NF-κB-inducing kinase
IKKα	NF-κB kinase subunit α
TGF	Transforming growth factor
GLI	Glioma-associated oncogene homolog

Contents

1 Introduction

Galectins are family of lectins with shared amino acid sequences, folding motifs and ability to bind β-galactosidase sugars through *N*-linked or *O*-linked glycosylation.

Up to this date 15 members of galectin family have been discovered with only galectin-1, -2, -3, -4, -7, -8, -9, -10, -12 and -13 found in humans. Galectin -5 and -6 are present in rodents and galectins -11, -14 and -15 are present in goats and sheep.

Each galectin has a carbohydrate recognition domain (CRD) and based on the structure and number of these CRDs we can distinguish three subgroups:

- Prototype galectins contain CRD and a short *N*-terminal domain, since they often create dimers with two identical CRDs the name dimeric galectins is also used. Galectin-1, -2, -5, -7, -10, -11, -13, -14 and -15 belong to the prototype or dimeric subgroup of galectin family.
- Tandem galectins have two different CRDs connected by a short linker, members of this subgroup are galectin-4, -5, -8, -9 and -12.
- Chimeric galectin is an assembly with one or even more units of the same CRD and unique amino terminus, only one member of this subgroup has been found to this date, galectin-3.

Galectins possess a variety of functions from the regulation of cell adhesion to the regulation of cell migration, cell growth, apoptosis and pre-mRNA splicing. Extracellular galectins bind *β*-galactosidase sugars located on the surface of the cell or in the extracellular matrix (ECM) and therefore, promote cell-cell interaction and cell-matrix interaction. These interactions are unique to every galectin based on the CRDs it contains. (Barondes, Cooper, Gitt, & Leffler, 1994; Ebrahim et al., 2014; Elola, Wolfenstain-Todel, Troncoso, Vasta, & Rabinovich, 2007)

The aim of this bachelor's thesis is to summarize mechanisms by which galectins promote cancer cell invasiveness and therefore, the formation of metastasis.

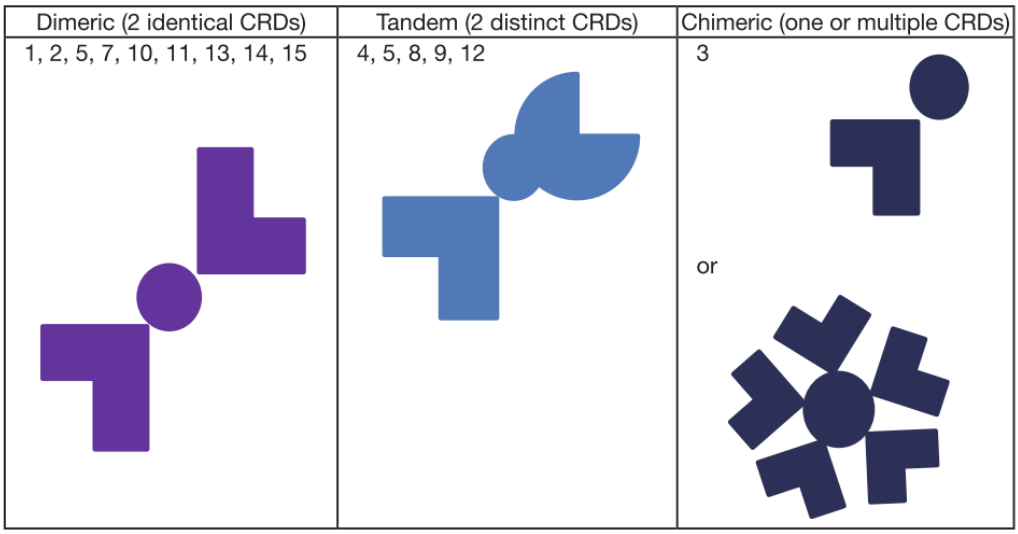

FIGURE 1: STRUCTURAL MODELS OF THREE SUBGROUPS OF GALECTINS

2 Galectins and cancer

Galectins appear to play many roles in tumorigenesis and tumour development, with often high expression levels in cancer cells. The pathogenic phenotype is promoted by sudden expression of galectins not commonly detectable in the specific cell type or by the lack of certain galectins that under normal conditions could be found in the cell line in question. (F. T. Liu & Rabinovich, 2005) Findings on galectins, and whether they promote or inhibit certain events vary a lot, probably due to the different signalling contexts in various cancer types. (Dumic, Dabelic, & Flögel, 2006) Cancer-related processes and the roles of galectins in the regulation of particular processes are listed below:

Neoplastic transformation is a process that healthy cells undergo to become cancer cells. Many mechanisms, when deregulated contribute to this transformation. One of them is caused by mutated oncogenic Ras protein, which for its proper function is required to be anchored in the plasmatic membrane. Both galectin-1 and -3 bind to the members of Ras family. Galectin-1 appears to mediate the anchorage of Ras itself while galectin-3 upon binding, preferentially K-Ras, initiates further signalling. (F. T. Liu & Rabinovich, 2005)

Angiogenesis is a process of creating new blood vessels. In case of tumour growth, delivering nutrition and creating a channel for cancer cells to disseminate. Galectins promote angiogenesis probably through their CRDs, which are capable of binding angiogenic factors or by creating a scaffold for the new blood vessels, by mediating interactions between epithelial cells and ECM. Galectin-1, in prostate cancer, Kaposi's sarcoma, melanoma and renal cell cancer, and galectin-3, in melanoma, induce the process of angiogenesis. (Cousin & Cloninger, 2016; Ebrahim et al., 2014; Takenaka,

Fukumori, & Raz, 2002) The complete opposite effect was shown for galectin-7, its ectopic expression appears to suppress this process in colon cancer. (Saussez & Kiss, 2006)

Apoptosis or programmed cell death is vital in every multicellular organism and suppressed in many types of cancer. Galectins regulate this process in various ways, based on their location. Not only does the type of cancer influence their effect but also whether the galectin is intracellular or extracellular. Certain galectins, namely galectin-7 and exogenous galectin-9 display pro-apoptotic effects on cells, therefore, acting negatively on tumour progression. Others such as galectin-3 protect the cell from programmed death and therefore, promote tumour growth. But even in case of this galectin, that is very often upregulated in cancer cells, its location in the nucleus seems to have the opposite effect to the cytosolic protein and promotes apoptosis. In addition, galectins participate in cancer development not only by protecting cancer cells from programmed death but also by inducing apoptosis of T cells, thereby avoiding an immune system response. Galectins-4 and -1 have been observed to modulate the immune system of the host in this way. (Cao & Guo, 2016; Cousin & Cloninger, 2016; Hirashima et al., 2004; F. T. Liu & Rabinovich, 2005; Saussez & Kiss, 2006; Takenaka et al., 2002)

Cancer cell migration consists of three events: cell adhesion, cell motility, and cell invasiveness. Galectins modulate all three of these processes. They promote adhesion either to ECM or the homotypic adhesion to other cancer cells. Namely galectin-9, -1 and -3 modulate cell aggregation, galectins-1 and -3 modulate adhesion to the ECM and galectin-4 and -3 were shown to induce adhesion of the cancer cells to the vascular endothelial cells. Galectin-1, -3 and -8 have been found to modulate, meaning promote or reduce, invasiveness depending on the cancer type. And finally, galectin-1 has been found to promote motility when upregulated, while the lack of galectin-4 seems to induce the same effect. (Bidon-Wagner & Le Pennec, 2002; Isabelle Camby, Le Mercier, Lefranc, & Kiss, 2006; Cao & Guo, 2016; Hirashima et al., 2004; F. T. Liu & Rabinovich, 2005; Takenaka et al., 2002)

Cancer cell growth is hard to assess, since increase or reduction of tumour growth can be due to many factors like already mentioned apoptosis or angiogenesis. Galectins-7, -4 and -8 are known to suppress tumour growth, even though galectin-9 is linked to good prognosis its effects are due to anti-metastatic features. While these above-mentioned galectins have mainly negative impact on cell growth, galectin-1 and -3 have more complex influence based on the type of cancer. Galectin-1 induces proliferation in various cell lines, such as spleen or lymph node, but also inhibits proliferation in for example neuroblastoma. In some types of cancer derived from astrocytic or colon tissues galectin-1 has no effect on tumour growth. Similar differences were observed for galectin-3 overexpressing tumours. Slower growth induced by galectin-3 has been demonstrated in the prostate, while in breast carcinoma cells or thyroid papillary carcinoma slower growth has been achieved by inhibiting galectin-

3, making galectin-3 the most potent tumour growth modulator from the galectin family. (Bidon-Wagner & Le Pennec, 2002; Isabelle Camby et al., 2006; Cao & Guo, 2016; Hirashima et al., 2004; F. T. Liu & Rabinovich, 2005; Saussez & Kiss, 2006)

2.1 Invasiveness and the role of galectins in it

Metastasizing, the process during which cancer cells detach from the primary site, invade through ECM, move through lymphatic or blood vessels and colonize the secondary site, accounts for more than 90% of cancer fatalities (for a recent review see (S. Li & Li, 2014)).

During dissemination of cancer cells from the primary site, these cells invade ECM either in clusters or sheets, which is called collective migration or as single cancer cells. Collective migration is dependent on secretion of proteolytic proteins to degrade the ECM on the leading edge of the cell and cell contractility. Single cell migration can use either a protease-dependent mesenchymal mechanism or a protease-independent ameboid mechanism or their combination. When changes in the environment occur many cells can switch between these two mechanisms based on their convenience. (Bronsert et al., 2014; Clark & Vignjevic, 2015; Friedl, Locker, Sahai, & Segall, 2012; Friedl & Wolf, 2003; Paňková, Rösel, Novotný, & Brábek, 2010)

Some galectins have a significant impact on cancer cell invasion due to their resemblance to matricellular proteins, acting as crosslinkers and therefore, modulating cellular interactions. Galectins just as matricellular proteins possess certain characteristics that can promote invasiveness and metastasizing: they don't play any structural roles but function contextually as modulators of cell-matrix interactions, they bind to many cell-surface receptors and generally modulate adhesion. (Bornstein & Sage, 2002)

Most galectins haven't been found to be overexpressed in invasive tumour cells, except galectins-1, -3 and -8. All three of them are potent in the modulation of cell migration, galectin-3 being the strongest and galectin-8 being the weakest. When comparison of expression was made between the invasive part and the central part of the tumours it was found that mostly galectin-1 and -3 contribute to invasiveness, with in case of galectin-1 the same concentration in central and invasive part and in case of galectin-3 with higher concentration in the invasive part of the tumour. Galectin-8 has been found to be expressed more in the central part (I Camby et al., 2001), therefore, from now on we will mainly focus on galectin-1 and galectin-3 and their role in invasiveness.

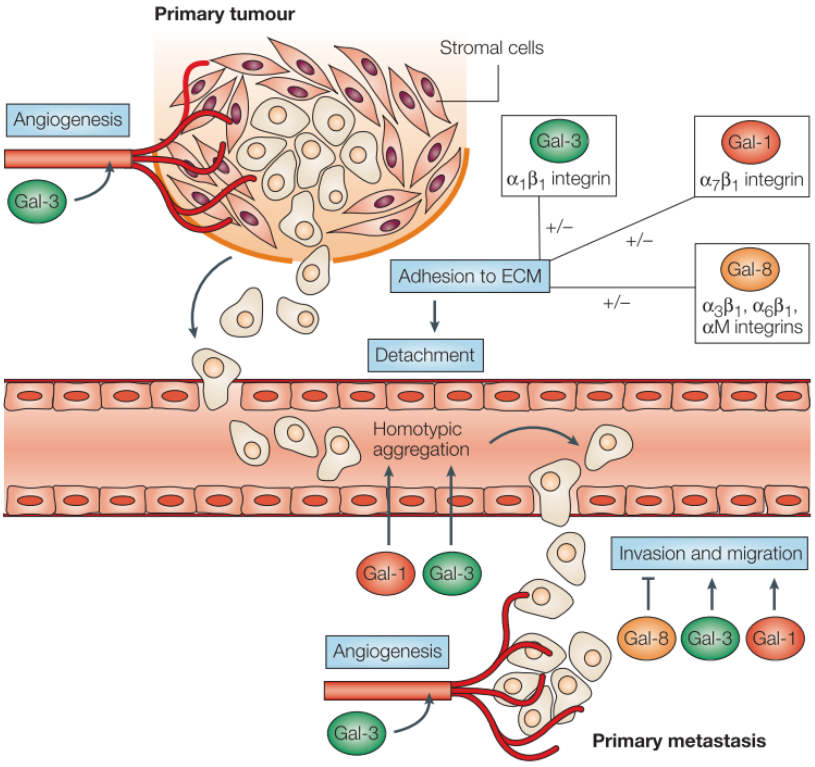

FIGURE 2: ROLES OF GALECTINS IN THE FORMATION OF METASTASIS (F. T. Liu & Rabinovich, 2005)

3 Gene and protein

3.1 Galectin-1

Galectin-1 is encoded by *LGALS1* gene, which is located on the 22q12 chromosome. It contains 4 exons and its transcript length is 0,6 kbp. (Chiariotti, Salvatore, Frunzio, & Bruni, 2002)

Galectin-1 belongs to the prototype group of galectins. The protein, 14,5 kDa in size, contains 135 amino acids and is folded into β-sandwich, which consists of two antiparallel β-sheets and six strands. Two identical monomers interacting at the interface create homodimer with CRDs on the far end of each monomer, 44Å distant from each other. The dimer is very stable, which is explained by hydrophobic core at the centre. (López-Lucendo et al., 2004)

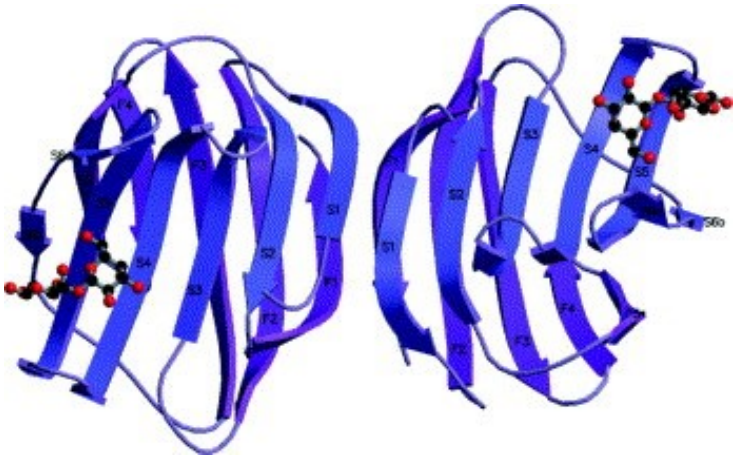

FIGURE 3: GALECTIN-1 PROTEIN, A TYPICAL FOLDING PATTERN OF GALECTINS CALLED "JELLY ROLL" (López-Lucendo et al., 2004)

3.2 Galectin-3

Galectin-3 is encoded by *LGALS3* gene located on the 14th chromosome, locus q21-q22. It contains six exons and five introns. (Dumic et al., 2006)

Galectin-3 protein is 31 kDa in size and is the only chimera type galectin in the galectin family. Its structure is very different from the other galectins as it consists of a single polypeptide with two functional domains. C-domain, which consists of 135 amino acids, and very long and flexible N-domain, 100-150 amino acids long, that appears to be unique, containing chain rich in proline, glycine, tyrosine and glutamine and missing charged or large side-chain hydrophobic amino acids, necessary for phosphorylation and further secretion of the protein. (Krześlak & Lipińska, 2004)

Just like galectin-1 galectin-3 is also composed of 5-stranded and 6-stranded β-sheets, but it does not possess the canonical symmetric dimer interface even though in certain conditions its valency

could be dynamic, meaning it could arrange itself into an oligomer in equilibrium with the monomer. (Seetharaman et al., 1998)

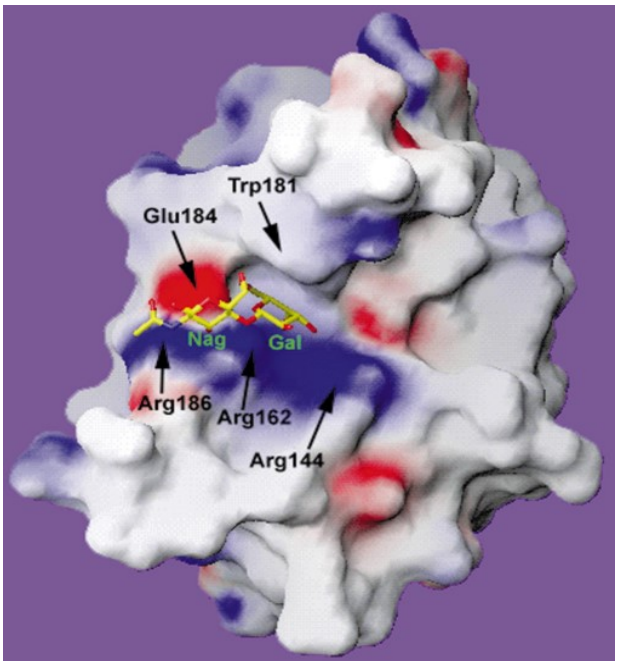

FIGURE 4: AN ELECTROSTATIC POTENTIAL OF GALECTIN-3 BINDING SITE, BLUE INDICATES NEGATIVE CHARGE AND RED POSITIVE

4 Function

4.1 Galectin-1

Galectin-1 was first identified as a lectin that binds to β-galactosides in the ECM. Later it was also documented to be involved in intracellular protein interactions. (Isabelle Camby et al., 2006) Galectin-1 binds a number of glycoproteins and glycolipids, the key is that the binding partners carry the right oligosaccharides. (Tinari et al., 2001) It is localized in a variety of tissues from skeletal, smooth and cardiac muscle, neurons to thymus, kidney, and placenta. Its function depends on the ligands interacting with galectin-1, but the most distinct role of galectin-1 is promotion or reduction of cell adhesion. (Barondes et al., 1994)

4.1.1 Intracellular function

Inside of the cell, galectin-1 acts as a scaffold protein for signalling pathways. (Cousin & Cloninger, 2016) There are several intracellular interactions relevant to cancer biology, the most important interacting proteins are listed below:

Among the most important interactors of galectin-1 is H-Ras. H-Ras promotes malignant transformation, its interaction with galectin-1 is essential for membrane anchorage of H-Ras and for its transforming activity. (Paz, Haklai, Elad-Sfadia, Ballan, & Kloog, 2001).

Protocadherin-24 (PCDH24) is another protein that is associated with galectin-1 and cancer. Its overexpression is linked to suppression of tumour growth and inhibition of cell proliferation. When PCDH24 is expressed β-catenin is localized in the cytoplasmic membrane. PCDH24 translocates β-catenin to the cell membrane, it has been found that regulation of this translocation happens *via* galectin-1. (Ose & Nagase, 2012)

The other well-documented interaction is the interaction of galectin-1 with Gem nuclear organelle associated protein 4 (Gemin4). Gemin4 is a component of macromolecular complex localized in the cytoplasm, with function in spliceosomal small nuclear ribonucleoproteins assembly. (J. W. Park, Voss, Grabski, Wang, & Patterson, 2001)

4.1.2 Extracellular function

In ECM multivalent properties of galectin-1 play an important role. Since galectin-1 occurs as a dimer in solution it has two binding sites and therefore, can provide a crosstalk between cells, and cells and associated stroma. (Cousin & Cloninger, 2016; López-Lucendo et al., 2004; Tinari et al., 2001)

Just like intracellular galectin-1 has multiple binding partners so does extracellular. The most important extracellular proteins, interacting with galectin-1 are listed below:

Laminin, a glycoprotein present in the base membrane, that separates endothelial and epithelial cells from connective tissue, is one of the binding partners of galectin-1 (Brûle et al., 2003), that takes part in a scaffolding of tissues. This heterotrimeric glycoprotein has been shown to play a big role in remodelling tumour angiogenesis, invasion, and metastasis. (Qin, Rodin, Simonson, & Hollande, 2017)

Another glycoprotein that has been found to be a binding partner of galectin-1 is fibronectin (Brûle et al., 2003). This protein could be found either in plasma, synthesized by hepatocytes or cellular, produced by the cells themselves. Fibronectin is vital for normal development, cell adhesion, migration, and homeostasis. In order for fibronectin to play its role, it has to be assembled into fibrils in ECM, that bind to molecules on the surface of the cell. (Maurer, Ma, & Mosher, 2015) Binding of galectin-1 to laminin and fibronectin promotes cell adhesion, furthermore, it has been suggested that on the border of tumours it could promote invasion. (Brûle et al., 2003)

MUC1/mucin(s) expressed in human trophoblast also interact with galectin-1, (Bojic & Vic, 2014) this interaction participates in trophoblast cell invasion (Kolundz & Vic, 2011).

The glycoprotein Mac-2 binding protein (MAC-2BP) with immunostimulatory activity binds to galectin-1 by lectin-carbohydrate interaction. Interaction between galectin-1 and glycoprotein MAC-2BP mediates cell aggregation, either by binding two glycoproteins MAC-2BP on surfaces of two different cells resulting in homotypic cell adhesion or by binding to secreted glycoprotein MAC-2BP, which is an oligomer, therefore, capable of binding more than just one galectin-1 molecule, and so creating a homotypic cell adhesion where glycoprotein MAC-2BP functions as so-called "bridge". It is not entirely conclusive which of these two methods are used even though the first option is more favoured. (Tinari et al., 2001)

4.2 Galectin-3

Galectin-3 has been localized in the intracellular and the extracellular environment just like galectin-1. Inside of the cell galectin-3 can be found not only in the cytoplasm, where it has multiple binding partners but also in the nucleus, where it enables the formation of spliceosomal complex and therefore, splicing of pre-mRNA (Dagher, Wang, & Patterson, 1995). Extracellular galectin-3, located either on surface of the cell or ECM, also binds multiple ligands and mediates cell adhesion and signalling. (Dumic et al., 2006) Galectin-3 is mainly found apart from epithelial cells and some sensory neurons in cells of the immune system such as activated macrophages, basophils, and mast cells, where due to binding IgE and IgE receptors, induces their activation and therefore, plays an important role in inflammation. (Barondes et al., 1994)

4.2.1 Intracellular function

Galectin-3 shares certain ligands with galectin-1, in the intracellular environment it is a component of a macromolecular complex, Gemin4. (J. W. Park et al., 2001) K-Ras a highly homologous protein to H-Ras is the only member of Ras superfamily that binds galectin-3 (Shalom-Feuerstein, Cooks, Raz, & Kloog, 2005), this interaction has been found to promote invasiveness (K. L. Wu et al., 2013) and the mechanism is discussed further at the later stage. The interactions that differ from galectin-1 are listed below:

ALG-2-interacting protein X (Alix), a regulator of protein transport and expression of some receptors on the surface of the cell, has been identified as a galectin-3 binding partner. (H.-Y. Chen et al., 2009) The interaction between Alix and galectin-3 regulates expression of epidermal growth factor receptor (EGFR) by intracellular trafficking, endocytosis of the receptors and their recycling. A concentration of epidermal growth factor receptors regulates keratinocyte migration and wound re-epithelization. In migrating cells Alix could be found on the leading front together with galectin-3 which suggest that this interaction could regulate translocation of more membrane proteins than just EGFR. (W. Liu et al., 2012)

Galectin-3 shares a certain domain called NWGR with B-cell lymphoma 2 protein (Bcl2). This domain is vital for the function of this protein. (Akahani, Nangia-Makker, Inohara, Kim, & Raz, 1997) Bcl2 is located at the mitochondrial membrane where it controls programmed cell death. (Vervloessem et al., 2017) This domain enables galectin-3 to replace Bcl2 in its antiapoptotic function (Akahani et al., 1997) and also this homology allows interaction between galectin-3 Bcl2, since both of these proteins have tendencies to self-associate. (Yang, Hsu, & Liu, 1996)

It binds a zinc finger cysteine- and histidine-rich cytoplasmic protein (Chrp) in a carbohydrate-independent manner, leaving the binding site free for polylactosamines like laminin. Also, the cysteine and histidine-rich domain of Chrp is left free for further interactions. Formation of this higher-order heterodimer might be important for the function of galectin-3 by mediating interactions of two different ligands one bound to galectin-3 and the other to Chrp. (Bawumia, Barboni, Menon, & Hughes, 2003)

Intermediate filaments are an element of the cytoskeleton, the role of these microfilaments is to maintain the shape of the cell under stress or mechanical force. This group could be divided into six subgroups, where I and II are made of cytokeratins. Keratins, intermediate filaments of epithelial cells, have a very complex structure made from fragments varying in size from 44 kDa to 66 kDa. (Pastuszak et al., 2015) Cytokeratins might be post-translationally modified into carrying a glycan with terminal linked *N*-acetylgalactosamine, which is recognized by galectins with type II recognition domain such as mammalian galectin-3. Since galectin-3 has a mRNA-splicing role in the nucleus this kind of anchorage by cytoplasmic protein might regulate its transport to the nucleus and therefore, the mRNA-splicing. (Goletz, Hanisch, & Karsten, 1997)

Another of galectin-3 binding partners is β-catenin (Shimura et al., 2004), a part of Wnt/ β-catenin signalling pathway. The concentration of β-catenin is regulated by the stimulation of Frizzled family receptors, if these receptors are stimulated by Wnt proteins they destroy the complex of adenomatous polyposis coli (APC), axin and glycogen synthase kinase 3 β (GSK β), which is responsible for phosphorylation of β-catenin. As a result, β-catenin concentration is increased, and it is free to translocate to the nucleus where it interacts with the transcription complex T-cell factor/lymphoid enhancer factor-1 (TCF/Lef1). After the activation of this complex, the target genes of this pathway can be transcribed. Wnt/β-catenin pathway has been linked to multiple processes of cancer pathogenesis. (Pai et al., 2017)

4.2.2 Extracellular function

Among the shared ligands with galectin-1 are laminin and fibronectin, the connection to laminin can be abolished by introducing lactose to the cell culture while the interaction of the cells

with fibronectin is not completely diminished by this, suggesting these interactions could be also provided by other particles such as integrins. (Matarrese et al., 2000)

Galectin-3 was discovered, even before galectin-1, to interact with MAC-2BP. Interaction with this heavily *N*-glycosylated protein mediates cell aggregation *via* creating bridges between cells as mentioned before. (Inohara, Akahani, Koths, & Raz, 1996; Rosenbergs, Cherayils, & Isselbacher, 1991)

Elastin, a protein produced by elastases binds to galectin-3 through its carbon recognition domain. (Ochieng, Warfield, Green-Jarvis, & Fentie, 1999) Elastin is a glycine-rich protein, where the concentration of glycine correlates with elasticity, is vital for organ and tissue elasticity and could be secreted into the bloodstream where it can recruit inflammatory cells and vasodilatation. (Debelle & Tamburro, 1999) Galectin-3 has been found to interact with soluble and insoluble elastin, it has been speculated that extracellular galectin-3 forms a complex with the precursor of elastin/laminin receptor and by doing so creates a mature receptor capable of binding cells to elastin. Furthermore, galectin-3 together with elastin can form a bridge between cells, some of the galectin-3 acting as part of the elastin/laminin receptor some interacting directly with the insoluble elastin. (Ochieng et al., 1999)

Another major participant in forming basement membrane interacting with galectin-3 is collagen IV. (Ochieng, Leite-Browning, & Warfield, 1998) This glycoprotein, built from six α-chains, creates a web-like scaffolding that further interacts with laminin. This scaffolding of basement membrane plays a big role in cell adhesion, migration, differentiation, and growth. (Tanjore & Kalluri, 2006)

Galectin-1 and -3 have many functions in cancer-related processes, they modify the regulation of cell growth, adhesion, motility and cell invasion they even influence activated T-cell survival. (Isabelle Camby et al., 2006; F. T. Liu & Rabinovich, 2005) It is not surprising that has a very significant part in cancer cell biology, further we will focus on cell invasion and the role these galectins play in it.

4.3 Galectins-1, -3 and invasiveness

There is a lot of experimental evidence for the role of galectin-1 and galectin-3 in cancer cell invasiveness.

Galectin-1 has been found to promote invasiveness in urinary bladder urothelial carcinoma (Shen et al., 2016), lung cancer tumour (Hsu, Wu, & Hung, 2013), glioblastoma (I Camby et al., 2002; Tousant III et al., 2012), ovary carcinoma (Brûle et al., 2003), cervical cancer (H.-J. Kim et al., 2013), oral squamous cell carcinoma (Chiang et al., 2008), prostate cancer (Tian et al., 2016), breast cancer (Zhu et al., 2016) and many others.

Galectin-3 has been observed to promote invasiveness in osteosarcoma (G. Bin Park et al., 2015), tongue cancer (Zhang et al., 2013), ovarian cancer (Mirandola et al., 2014), hepatocellular carcinoma (Serizawa et al., 2015), lung cancer (O'Driscoll et al., 2002), pancreatic cancer (Shumei Song et al., 2012), melanoma (Wang et al., 2012), gastric cancer (S. J. Kim et al., 2010), meningioma (Ahmed, Shebl, & Habashy, 2017), colon cancer (K. L. Wu et al., 2013), thyroid cancer (J. Zheng et al., 2017), prostate cancer (Meng, Joshi, & Nabi, 2015) and others.

The discovered mechanisms by which galectins alter the invasiveness of cancer cells are listed and described below:

4.3.1 Promoting invasiveness through upregulation of matrix metalloproteinases

Matrix metalloproteinases (MMP) are zinc-depended enzymes involved in tumour metastasis. (Nabeshima, Inoue, Shimao, & Sameshima, 2002) To this date we recognize 25 peptidases belonging to this family, these could be categorized into six subgroups depending on the substrate they cleave. (Wieczorek, Wasowicz, Gromadzinska, & Reszka, 2014) MMP-2 and MMP-9, the proteases that are upregulated the most in invasive cancer cells, belong to the subgroup of gelatinases. (M.-H. Wu et al., 2009) On top of their physiological function, degradation of components of ECM as collagen, gelatin, proteoglycan etc., these enzymes can modify cell-cell and cell-ECM signals. (Wieczorek et al., 2014)

Multiple pathways modified by galectin-1 and galectin-3 have been observed to increase expression of mainly MMP-2 and MMP-9.

MAPKs (mitogen-activated protein kinases) are evolutionarily conserved enzymes connecting cell-surface receptors to critical regulatory targets within the cell. Their activity is regulated by three-tiered cascades composed of a MAPK, MAPK kinase (MAPKK, MKK or MEK) and a MAPKK kinase or MEK kinase. Activity of these enzymes can be modulated by small GTP-binding proteins like Ras

subfamily. Mammals express multiple distinctly regulated groups, the one of our interest is Jun amino-terminal kinases (JNK1/2/3) that are activated by MKK4/7. (Chang & Karin, 2001)

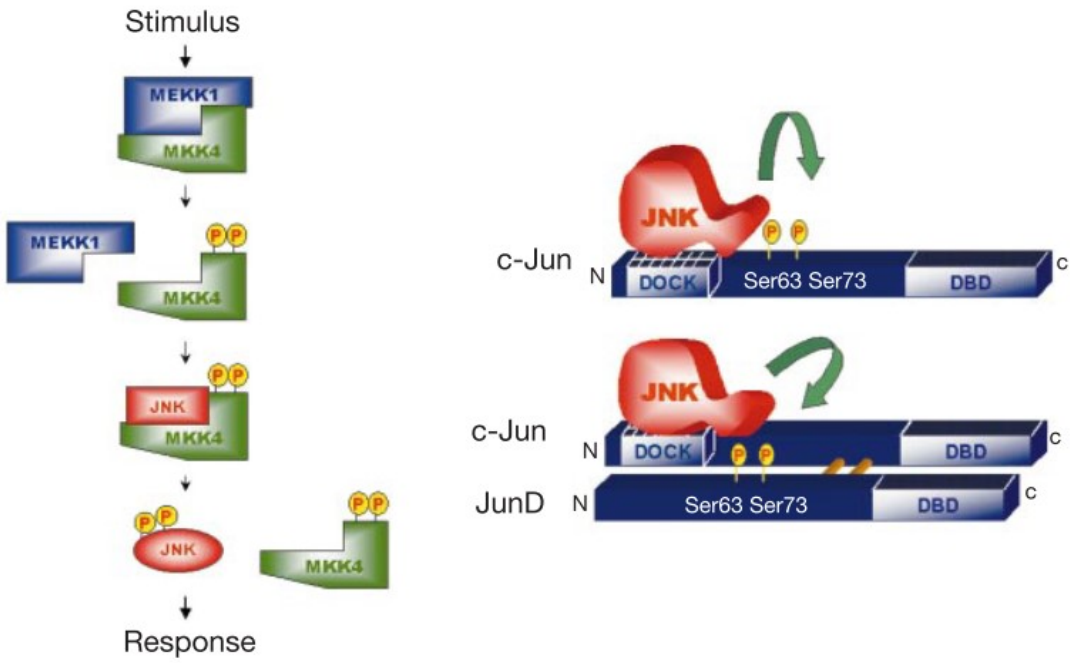

FIGURE 5: LEFT – SEQUENTIAL AND SPECIFIC INTERACTIONS BETWEEN MEMBERS OF THE CASCADE. RIGHT – JNK BINDS TO DOCKING REGION, WHICH SECURES PHOSPHORYLATION AT SER 63 AND SER 73, IF A SUBSTRATE LACKS THIS SITE PHOSPHORYLATION IS IMPOSSIBLE (JUND)(Chang & Karin, 2001)

Galectin-1 interacts with this signalling cascade by inducing H-Ras membrane anchorage and therefore, translocation, which is vital for Ras activity. H-Ras then probably activates Rac1 which interacts with MEKK4 and therefore, influences the whole MAP cascade. Cells with knock out of galectin-1 had reduced levels of c-Jun and consequentially blocked binding of activator protein 1 (AP1) to MMP-9 promoter and decreased expression of MMP-9 (Zhu et al., 2016). From these results we can assume that galectin-1 regulates Ras -Rac1-MEKK4-JNK-c-Jun-AP1 signalling pathway and as a result the level of MMP-9 (Shen et al., 2016).

The nuclear factor-κB (NF-κB) is a family of transcriptional factors found in all kinds of animal species and in nearly every cell type. These transcription factors are vital for development, regulation of immune system through Toll-like receptors, inflammation and wound healing. In most cells, NF-κB is present in its inactive state and only initiate its translocation to the nucleus after activation. It consists of five members p50, p52, p65, Rel-B, and c-Rel. These molecules form homo- and heterodimers with each other some with higher frequency than others. To this day there were described two main NF-κB signalling pathways: canonical and non-canonical pathway. In canonical pathway heterodimers, p50 and p65 are bound to the B-cells inhibitor α (IκBα) in the cytoplasm. The

heterodimer is released from this bond after phosphorylation of IκBα by the inhibitor of NF-κB kinase complex (IKK) and free to be translocated to the nucleus and act as a transcriptional factor. For this signalling to take place IKK must be activated by phosphorylation through one of the receptors that act as upstream effectors of the NF-κB pathway. Much less is known about the non-canonical pathway, where heterodimer p100 and Rel-B must be phosphorylated to turn into heterodimer p52 and Rel-B. For this process, NF-κB-inducing kinase (NIK) and NF-κB kinase subunit α (IKKα) is required. (Espín-Palazón & Traver, 2016; White et al., 2011) Expression of MMP-2 and MMP-9 is altered through NF-κB (M. H. Park, Ahn, Hong, & Min, 2009), which is possibly regulated by protein kinase B (Akt) *via* mTOR and Raptor (Dan et al., 2008).

A lower concentration of molecules participating in the canonical NF-κB pathway has been detected in galectin-1 knockdown cells, specifically p65 and IKKα. This suggests that galectin-1 downregulates NF-κB pathway and in result production of MMP-2 and MMP-9, as a downstream target of these transcriptional factors. (L. Chen, Yao, Sun, & Tang, 2017)

Galectin-3 has also been found to increase expression of MMP-2 and MMP-9, through upregulation of focal adhesion kinase (FAK). Activated FAK then activates proto-oncogene tyrosine-protein kinase Src (Src) and Lck/Yes novel tyrosine kinase (Lyn), which induce stimulation of phosphoinositide 3-kinase (PI3K) and Akt. (G. Bin Park et al., 2015). Akt phosphorylates and therefore, inactivates GSK-3β, protein responsible for degradation of β-catenin (S Song et al., 2009; Zhang et al., 2013), which has been proven to take part in promoting cancer cell invasiveness either by upregulating MMPs or promoting EMT (Lin et al., 2017; Peng et al., 2017; X. Tang et al., 2017) β-catenin translocates to the nucleus where it interacts with TCF/Lef1 transcription complex (Pai et al., 2017). MMP-7 has been proven and other MMPs have been predicted to have TCF/Lef1 binding sites, therefore, they could be upregulated by overexpression of β-catenin. (Brabletz, Jung, Dag, Hlubek, & Kirchner, 1999) MMP-2 and MMP-9 have been observed to be the targets of FAK/Src pathway through PI3K/Akt/ β-catenin pathway together with proinflammatory cytokines such as vascular endothelial growth factor (VEGF), monocyte chemoattractant protein-1 (MCP-1), interleukin 8 (IL-8) and interleukin 6 (IL-6). Expression of these cytokines seems to be upregulated by FAK/Src/Lyn pathway, in this case not by activation of PI3K/Akt but through the activation of the extracellular signal-regulated kinase (Erk). (G. Bin Park et al., 2015) Galectin-3 mediated β-catenin upregulation has been well observed and established mechanism in various cancer tissues from pancreatic cancer (Kobayashi et al., 2011), tongue cancer (Zhang et al., 2013) to osteosarcoma (G. Bin Park et al., 2015).

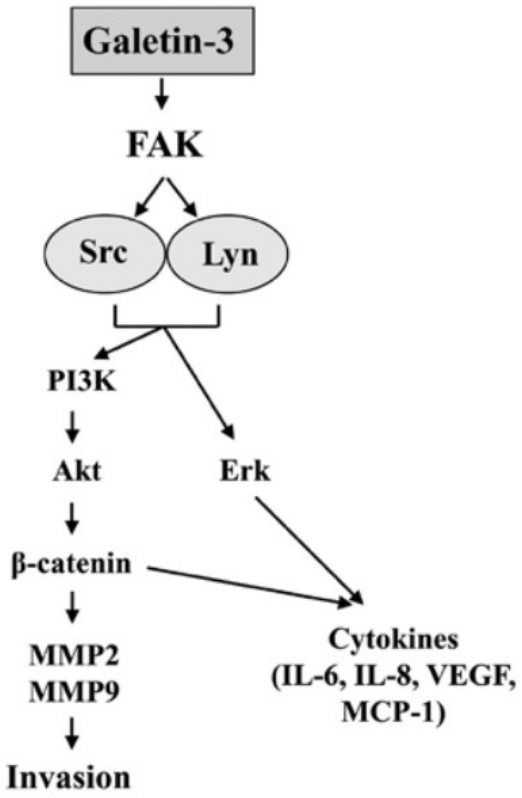

FIGURE 6: SIGNALLING PATHWAY LEADING TO OVERPRODUCTION OF METALLOPROTEINASES-2 AND -9 (G. Bin Park et al., 2015)

K-Ras, a GTP-binding protein, is one of the members of The Ras superfamily together with H-Ras and N-Ras, which are highly active in tumours. Even though these proteins are highly homologous, they have been proven to control different processes with K-Ras being expressed in all cell types. In its active state this protein binds GTP which is hydrolysed by GTPase activating protein (GAP), GDP is then exchanged by guanine nucleotide exchange factor (GEF). Essential for activation of these proteins is their localisation to the inner side of the plasma membrane, which makes the recruitment of the target enzymes possible. (Downward, 2003) Galectin-3 enhances K-Ras activity and its downstream signalling of Raf/Erk1/2, (K. L. Wu et al., 2013) perhaps by binding and keeping K-Ras at the plasma membrane. (Shumei Song et al., 2012) Another possible mechanism by which galectin-3 upregulates activation of K-Ras is by binding K-Ras and therefore, stabilizing the K-Ras-GTP complex. Supporting this claim is the observation that galectin-3 downregulates activation of N-Ras, which is not a direct binding partner of galectin-3. Perhaps by binding K-Ras and protecting its association with GTP, GAP enzymes are free to hydrolyse greater amount of N-Ras. Due to this mechanism, phosphorylation of Erk is increased. (Shalom-Feuerstein et al., 2005) One possible target of this signalling pathway promoting invasiveness could be the expression of MMPs (Downward, 2003), even though it has been proven that Ras can

cause upregulation of MMPs through this pathway, studies connecting galectin-3 to my knowledge have not yet been conducted.

Galectin-3 upregulates not only the expression of MMP-2 and MMP-9, but also MMP-1 and even though the mechanisms of this upregulation have not yet been elucidated, co-expression of MMP-1 and protease activated receptor-1 (PAR-1) has been observed. MMP-1 activates PAR-1 (S. J. Kim et al., 2011) together with other molecules such as thrombin, by cleaving the receptor. PAR-1, a transmembrane G-protein-coupled receptor, is just like MMPs, expressed in highly invasive cells, where it promotes invasiveness through multiple mechanisms. It inhibits apoptosis and increases expression of adhesion proteins, such as integrins, through which FAK is activated. Results even suggest that activation of PAR family receptors may lead to induction of EMT. (Wojtukiewicz, Hempel, Sierko, Tucker, & Honn, 2015)

4.3.2 Promoting invasiveness through re-organization of the actin cytoskeleton

Migration induced by actin cytoskeleton reorganization is vital for cell invasiveness. It is mediated through the assembly of actin, organized into three-dimensional shapes called lamellipodia and filopodia. Lamellipodia are sheet-like shaped, containing branched net of actin and are usually the base for finger-like shaped filopodia, which contain thin stacks of F-actin that sprout out of them. These structures are very dynamic and are continuously adjusted based on the needs of the cell. (Mattila & Lappalainen, 2008) These adjustments are regulated by Rho family small GTPases such as Rac, cell division control protein 42 homolog (Cdc42) and Rho. Each of them has a different role in the process of creating the lamellipodia, adhesion of the lamellipodia to the surface, the movement of the cell itself and retraction of the trailing end. (Yamazaki, Kurisu, & Takenawa, 2005)

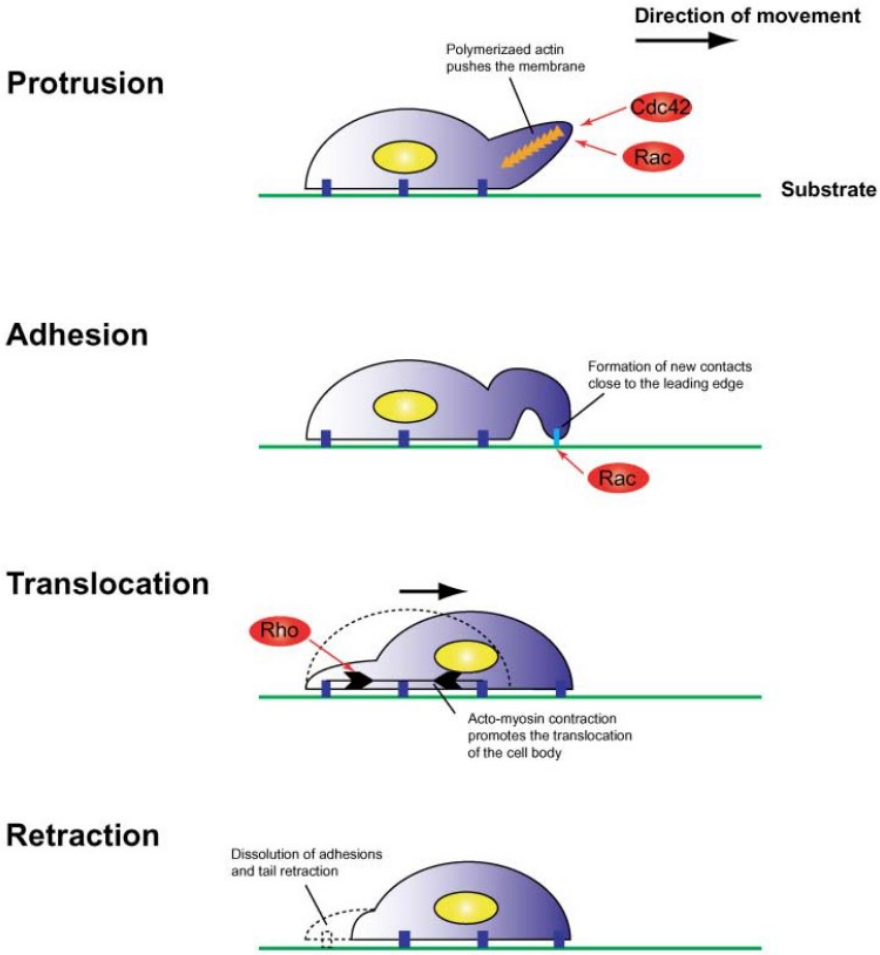

FIGURE 7: MIGRATION OF A CELL. RAC AND CDC45 INDUCE THE FORMATION OF LAMELLIPODIA AND FILOPODIA ON THE LEADING SIDE AND RHO RETRACTS THE LAGGING SIDE. (Yamazaki et al., 2005)

Galectin-1 acts as an upstream regulator of these GTPases, it increases the density of filopodia by 60% and their length by 45%. (M.-H. Wu et al., 2009) Ras homolog gene family member A (Rho-A) described before as a modulator of actin polymerisation is overexpressed by 34% to 75% in cells treated with a galectin-1 solution, consequentially the motility of the cells increased by the maximum of 30%. (Isabelle Camby et al., 2002) Rho-A regulates the actomyosin system, cells that overexpress this GTPase have strongly scattered shape, pointy edges and start to spread with lamellipodial protrusions. (Yoshioka, Nakamori, & Itoh, 1999) We can observe the same trend with Cdc42 for galectin-1 treated cells, meaning that galectin-1 could promote Rho-A and Cdc42 activity, therefore, the rearrangement of the actin cytoskeleton and as a result elevate cancer cell migration and invasiveness. (M.-H. Wu et al., 2009)

Fascin, an actin-binding protein, is present at a very low concentration or even absent in healthy epithelial cells while in cancer cells its expression is heavily upregulated. (Hashimoto, Skacel, & Adams, 2005) In these cells, it can establish invasive and migratory phenotype through the formation

of filopodia. The highest concentration of fascin has been found on the invasive front of tumours, where it promotes degradation of basement membrane and dissemination. (Vignjevic et al., 2007) Galectin-3 upregulates synthesis of fascin through Wnt/β-catenin pathway, as fascin genes are one of the target genes of this pathway. Interaction proven in this case has been that between galectin-3 and GSK-3β, galectins-3 serine 96 has been identified as a binding site for this interaction. (S. J. Kim et al., 2010)

Myosin II, a two-headed motor, consisting of two types of chains light and heavy, is responsible for contraction and plays a big role in migratory processes. Myosin is activated by phosphorylation of Ser19 on the light chain, which allows myosin to interact with actin. Several enzymes take part in regulating this process. Myosin light chain kinase (MLCK) is responsible for phosphorylation and therefore, activation of myosin filaments, myosin phosphatase (MYPT1) on the other hand dephosphorylates myosin and deactivates it. Upregulation of myosin activity can be done not only by MLCK but also by inhibition of MYPT1, this process is mediated by Rho-associated protein kinase (ROCK) (Fumio Matsumura, 2005), which can also directly phosphorylate myosin light chains (F Matsumura, Totsukawa, Yamakita, & Yamashiro, 2001). Similar to galectin-1 (M.-H. Wu et al., 2009) galectin-3 has been also observed to induce RhoA activity. Intracellular galectin-3 in an autocrine manner induces RhoA/ROCK pathway that leads to myosin light chain phosphorylation and as a result to actin rearrangement. (Serizawa et al., 2015)

4.3.3 Promoting invasiveness through integrins and FAK

Integrins are extracellular receptors that consist of two noncovalently bound units α and β, with extracellular, transmembrane and cytoplasmic domain. (Schaffner, Ray, & Dontenwill, 2013) Integrins take part in two modes of signalling that are essential for invasion and migration, outside-in signalling, where they transfer outside stimulus and regulate intracellular signalling pathways and inside-out signalling, where they ensure adhesion to the ECM. (Hood & Cheresh, 2002)

FAK, a cytoplasmic kinase located in focal adhesions, adhesion structures that interact with ECM, and in the proximity of integrins, ensures integrin-mediated signalling upon cell adhesion. (Hood & Cheresh, 2002) After stimulation, FAK is autophosphorylated on Tyr-397, which has been also identified to be the site for Src homology 2 binding, necessary for further downstream signalling. (Schaller et al., 1994) Overexpression of FAK is often reported in highly invasive cells, and FAK was shown to promote invasiveness (Owens et al., 1995), this could be achieved through multiple signalling pathways in which FAK participates. Upregulation of FAK/Erk1/2 pathway leads to overproduction of MMPs and urokinase-type plasminogen activator, another signalling pathway that results in overexpression of MMPs in which FAK plays a key role is FAK/PI3K/Akt/mTOR (Neoh et al., 2017). FAK also has a regulatory effect on small GTPases through which it remodels actin cytoskeleton and

promotes EMT, a process of morphological transformation required for cell invasion and migration. (Tai, Chen, & Shen, 2015)

Integrin α6β4, a laminin-binding receptor involved in hemidesmosome organisation primarily located in the basal surface of epithelia, has recently been associated with carcinoma cell migration. (Mercurio, Rabinovitz, & Shaw, 2001) α6β4 integrin acquires a new role in invasive cells that is drastically different from the one in epithelial cells where it anchors cells to basement membrane through interactions with cytokeratins (Mercurio & Rabinovitz, 2001), it is overexpressed in certain carcinomas and reassigned from hemidesmosomes to migratory parts of the cell. (Hood & Cheresh, 2002) Overexpression of α6β4 has been found to be induced in more aggressive thyroid tumours, where it can enhance invasiveness since α6β4 is more versatile laminin receptor capable of mediating bond with laminin that leads to tissue invasion than α3β1 integrin expressed in non-invasive cells. (Serini et al., 1996) This integrin has also a part in dynamic processes of migration, it participates in formation and stabilization of filopodia and lamellae, where it is associated with actin. (Rabinovitz & Mercurio, 1998)

It has been reported that galectin-1 upregulates both integrin α6β4 and signalling *via* FAK kinase. Increased FAK activity results in EMT and Akt activation. This effect caused by galectin-1 overexpression can be reversed by integrin α6β4 knockdown, suggesting that integrin is the key part of this invasion promoting pathway. Interaction of α6β4 integrin with certain tyrosine kinase receptors ensures autophosphorylation of FAK and therefore, phosphorylation of Akt which is a kinase with an important role in cancer invasion and migration. (Hsu et al., 2013)

The α5β1 integrin has high binding specificity for fibronectin, migration on fibronectin is insured by degradation of this integrin in lysosome. (Schaffner et al., 2013) Integrin α5β1 has been found to be overexpressed in highly invasive cells, where it increases adhesion, focal adhesion assembly, stress fiber formation and contractile forces. (Mierke, Frey, Fellner, Herrmann, & Fabry, 2011) β1 integrin-induced demethylation of *Lgals3* promoter has been observed, it therefore, epigenetically influences transcription of galectin-3 gene. In return galectin-3 enhances adhesion of these integrins to fibronectin and laminin, establishing a feedback loop that promotes cell migration. (Margadant, Van Den Bout, Van Boxtel, Thijssen, & Sonnenberg, 2012)

Caveolin-1 (Cav-1), a 22 kDa protein, is mainly located in plasmatic membrane, where due to its oligomerising ability acts as a coat for caveolae. With its scaffolding domain Cav-1 also interacts with certain signalling molecules such as G-proteins, receptor and non-receptor tyrosine kinases. Among these Rho, ROCK and FAK kinases are activated by upregulation of Cav-1 . (D. Chen & Che, 2014) Invasiveness promoting effect of Cav-1 can be induced by exogenous galectin-3. Galectin creates a

lattice together with mannosyl (α-1,6)-glycoprotein β-1,6-*N*-acetyl-glucosamintransferase (Mgat5), this association causes integrin clustering on the plasma membrane. Integrins then mediate tyrosine phosphorylation of Cav-1, which allows it to recruit FAK, paxillin and α5-integrin into the focal adhesions and increase their stability, therefore, induces signalling through these molecules. (Goetz et al., 2008) Addition of galectin-3 restores motility of prostate cancer cells after induced expression of polymerase I and transcription release factor (PTRF). PTRF decreases invasiveness probably by building Cav-1 into the membrane, while loss of PTRF results in non-caveolar scaffold domain Cav-1 location. Main location of Cav-1 in plasmatic membrane is linked to lower cancer migration, due to higher FAK destabilisation. Creation of galectin-3 and Mgat5 lattice reverses this effect by already mentioned mechanism. (Meng et al., 2015)

4.3.4 Promoting invasiveness through epithelial-mesenchymal transition

EMT is a process by which epithelial cells lose their cell polarity and cell-cell adhesion and gain migratory and invasive properties to become mesenchymal cells. E-cadherins, claudins, occludins and cytokeratins are downregulated while vimentin, fibronectin, fibroblast-specific protein-1, α-smooth muscle actin, N-cadherin and collagen I are upregulated. These changes trigger loss of adhesion junction in between cells, modification of actin cytoskeleton, loss of cell polarity and spindle-like shape of the cells, which all allow the cell to escape the primary site and invade surrounding tissue. EMT is regulated by multiple repressors and promoters on level of epigenetic modification, transcription, translation and subcellular localization. The most recognised promoters of mesenchymal state are members of transforming growth factor (TGF) superfamily and Twist, a transcription factor. The repressors of epithelial state are mainly zinc finger proteins SNAIL and ZEB. An important role in the process also play microRNAs. We can differentiate three subtypes based on the conditions EMT occurs in: 1. EMT associated with embryo implantation 2. EMT associated with organ fibrosis, translocation of cell for wound healing purposes 3. EMT associated with cancer metastasis, alteration of proteasome of the cell and promoting invasiveness. This latter conclusion is supported by findings that EMT occurs at the tumour invasive front. Multiple studies have found that invasiveness correlates with EMT, occurring in the early stage of the transition. Cells that undergo EMT can more efficiently invade surrounding tissue benefiting from the capability of repairing themselves, surviving under stress and differentiation. (Sung, Kim, & Park, 2016; D. Tang et al., 2017; Zou, Liu, Gong, Hu, & Zhang, 2017)

Both extracellular and intracellular galectin-1 has been found to induce EMT by multiple distinguished mechanisms.

Increased expression of galectin-1 induces a transition from epithelial to mesenchymal cell type. As mentioned before higher levels of protein SNAIL and vimentin, intermediate filaments with

an impact on cytoskeleton alterations, have been detected. Also, downregulation of E-cadherin has been reported most probably through PI3K/Akt signalling pathway. (Bacigalupo et al., 2015)

Pancreatic stellate cells (PSC) in their deactivated state contain excess vitamin A in a form of droplets. They can transform to activated state if exposed to oxidative stress, alcohol etc.. If activated PSCs can display a number of features such as promotion of cell proliferation, ECM protein synthesis, MMPs synthesis and many others. (Apte, Pirola, & Wilson, 2012) One of these proteins secreted by PSCs is also galectin-1. These cells interact with pancreatic cancer cells and promote EMT, by upregulating NF-κB and Twist, both transcription factors linked to epithelial-mesenchymal transition. As in the previous case, vimentin was observed to be upregulated and E-cadherin downregulated, in addition, higher production of MM9 was observed. (D. Tang et al., 2017) Higher production of galectin-1 by PSC is induced by transforming growth factor β1, which is secreted by pancreatic cancer cells creating a signalling cycle between these two cell types. (D. Tang et al., 2014)

The hedgehog highly conserved signalling pathway has been found to play role in EMT. (Ohta et al., 2009) It is initiated by binding of one of three ligands Sonic hedgehog, Indian hedgehog or Desert hedgehog to the Patched (PTCH) receptor, which consists of 12-transmembrane segments. PTCH acts as a repressor for Smoothened (SMO), the repression is cancelled by the creation of a bond between ligand and receptor. Once SMO is released it activates glioma-associated oncogene homolog (GLI) signalling cascade by inducing MAP3K10 activation and SUFU inactivation, this stabilizes GLI, a zinc finger transcription factor, and ensures its location to the nucleus. This mechanism is characteristic for hedgehog canonical signalling pathway. Non-canonical signalling is not as well defined, it is a pathway that doesn't respond to the impulses through Hedgehog-to-GLI route. We can distinguish three subtypes of non-canonical signalling: 1. Signals that originate at the PATCH receptor 2. SMO-depended activation 3. SMO-independent activation. (Katoh & Katoh, 2008; Teperino, Aberger, Esterbauer, Riobo, & Pospisilik, 2014) Intracellular galectin-1 modulates non-canonical SMO-independent signalling, which results in EMT. Overexpression of galectin-1 increases the level if GLI-1 but the SMO expression maintains its levels. The steady level of SMO could be explained by the feedback loop, when galectin-1, as mentioned before, interacts with Ras, production of Sonic hedgehog is increased which leads to cancelation of repressing effect of PATCH to SMO, but the presence of Sonic hedgehog negatively reflects on SMO production. As a result of this interaction GLI-1, which acts as a transcription factor promotes transcription and later translation of EMT genes. (Chong et al., 2016) One of these EMT promoting genes expressed during this process is epithelial repressor SNAIL. (X. Li et al., 2006)

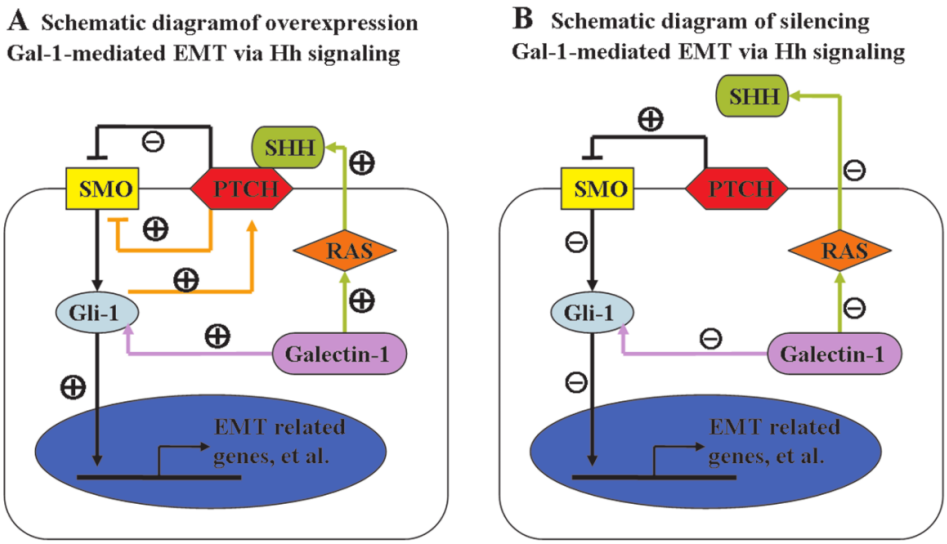

FIGURE 8: HEDGEHOG SIGNALLING PATHWAY AND THE NEGATIVE LOOP REGULATING SMO PRODUCTION

(Chong et al., 2016)

4.3.5 Regulation of invasiveness through uPAR expression

Urokinase-type plasminogen activator receptor (uPAR) augments ECM degradation, cell-ECM interactions, and cell signalling by binding urokinase-type plasminogen activator (uPA) and pro-urokinase-type plasminogen activator (pro-uPA) to the surface of the cell. uPA cleaves zymogen plasminogen to plasmin, a protease that cleaves a number of ECM elements together with pro-uPA, therefore, establishing a positive loop. uPAR is highly expressed during ECM remodelling such as during gestation, embryo implantation and wound healing. Plasminogen activation, therefore, uPAR expression, enables cells migration in ECM, also due to plasmins ability to cleave and activate MMPs. uPar doesn't promote migration and invasion only through activation of plasmin but also through downstream signalling, uPAR is an upstream regulator of many signalling pathways such as Ras/MAPK, FAK/Src, small GTPase Rac or PI3K/Akt (Smith & Marshall, 2010) Expression of uPAR is regulated by MEK/Erk pathway. (Bessard, Frémin, Ezan, Coutant, & Baffet, 2006) This pathways upstream regulator is Ras (Smith & Marshall, 2010), protein which binds and whose activity is highly affected by galectin-3 (K. L. Wu et al., 2013). It has been proven that galectin-3 promotes expression of uPAR and therefore, invasiveness through Ras/Erk pathway. Through overexpression of uPAR, galectin-3 achieves higher phosphorylation of Akt, member of PI3K/Akt signalling and downstream effector of uPAR signalling. (D. Zheng et al., 2014) PI3K/Akt signalling has been proved to play a big role in cancer invasiveness (Shukla et al., 2007), by different processes, for example, this pathway can lead to overexpression of MMP9 (J. S. Chen et al., 2009) or to overexpression of EMT proteins that leads to EMT itself (Baek et al., 2017).

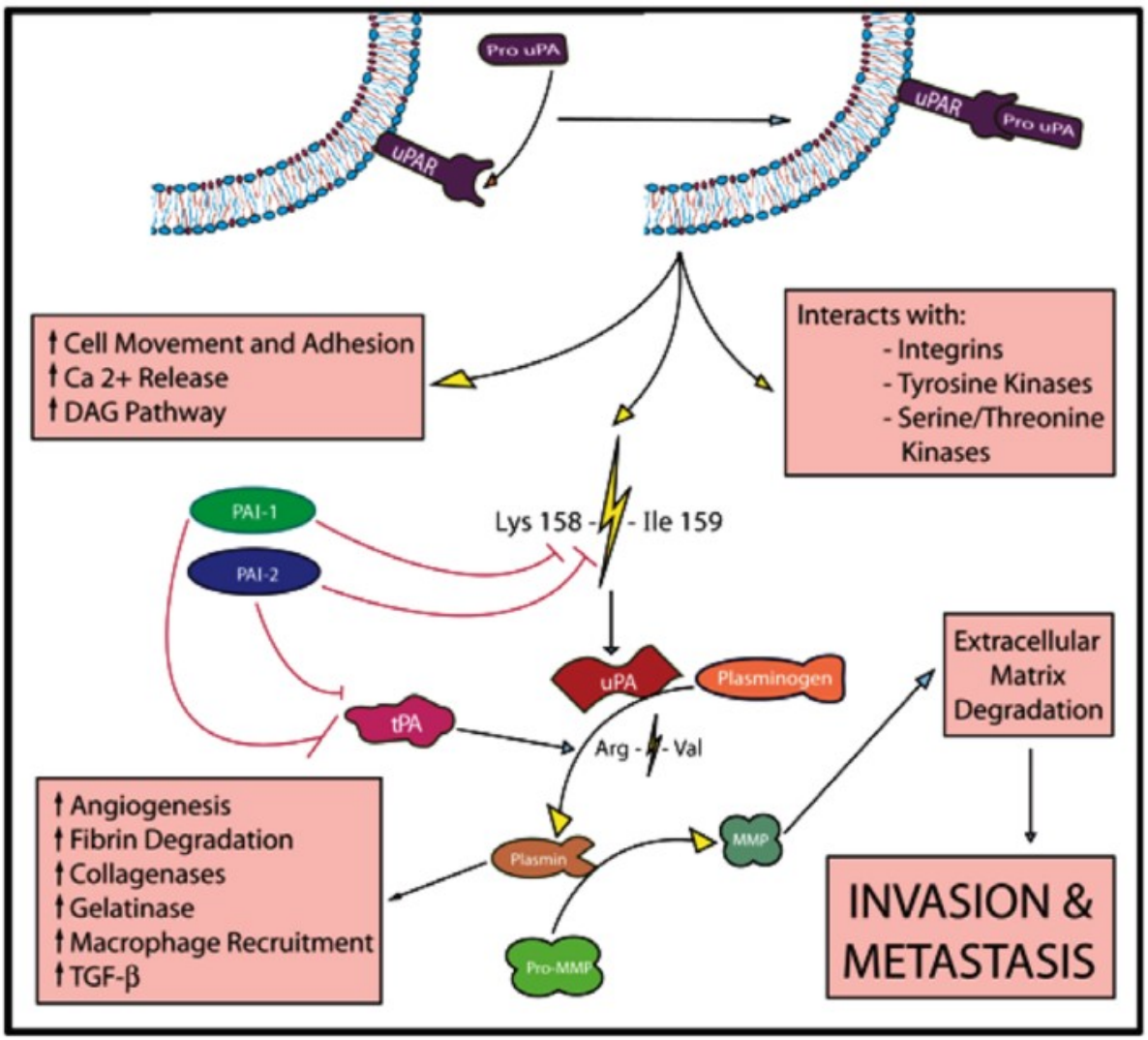

FIGURE 9: UROKINASE-TYPE PLASMINOGEN ACTIVATOR RECEPTOR SYSTEM (Dass, Ahmad, Azmi, Sarkar, & Sarkar, 2008)

4.3.6 Reducing invasiveness

As mentioned before galectins have a different effect on cancer progression processes based on the type of cancer. Even though galectin-1 and -3 have been proven to be mostly promoting invasiveness of cancer cells in some cases they have an opposite effect. Their exogenous forms have been observed to suppress migration of colon cancer cells. This effect might be due to the ability of galectins to mediate or inhibit cell-ECM interaction. (Hittelet et al., 2003) Galectin-3 has also been shown to reduce migration of breast cancer cells, by suppressing EMT, therefore, ensuring an epithelial form of the cells. (Ilmer et al., 2016) In glioblastoma cells, downregulation of galectin-3 expression results in promoting motility on the laminin coated surface but does not influence invasive or adhesive abilities of the cell. This was probably caused by an increase of expression of α6β1 integrin, a laminin-binding molecule, by unknown mechanism. (Debray et al., 2004)

5 Galectins as potential targets in cancer treatment

As already mentioned, galectins-1 and -3 are well established to play a major role in cancer progression. It is thus only logical that galectins became subject of many studies, related to anticancer therapy. Some of these studies have reported decreased invasiveness and motility, due to inhibition of particular galectins.

Sulforaphane-cysteine (SFN-Cys), a metabolite of isothiocyanate sulforaphane, appears to successfully inhibit invasiveness of prostate cancer cells together with cell proliferation and tumour growth. It does so by downregulation of galectin-1 expression through inhibition of Erk1/2 signalling pathway, thereby regulation of transcriptional factors such as AP-1. (Tian et al., 2016)

Naturally occurring Thomsen-Friedenreich (TF) antigen, a disaccharide, isolated from Pacific cod inhibits lung metastasis of prostate cancer cells. TF is often expressed by cancer cells, specifically on their surface, due to its ability to bind galectin-3 located on the surface of capillary endothelial cells and therefore, promoting cell migration. Addition of TF acquired from Pacific cod inhibits this process by binding to galectin-3 and prevents it to interact not only with TF on the surface of cancer cells but also with other ligands. (Guha et al., 2013)

Modified pectin (MCP), a hydrolysed water-soluble fiber, inhibited metastasis into lungs and reduced metastases to lymph nodes and liver from colon carcinoma cells and breast cancer cells implanted into nude mice. MCP binds to the matrix or cell surface located galectin-3 and inhibits its function, binding to endothelial cells and reducing the invasion of secondary sites. By binding to galectins CRD it inhibits all signalling, where galectin-3 binds its ligand through this domain, resulting not only in decreased invasiveness but also decreased angiogenesis and tumour volume reduction. (Nangia-Makker, Hogan, & Honjo, 2002)

Galectin-3C is a product of bacterial collagenase and recombinant human galectin-3. This shortened form of galectin-3 doesn't possess the cross-linking ability of the full-length one, therefore, competing for ligands with galectin-3 but failing to carry out functions of full-length galectin. It inhibits metastasis and reduces tumour growth of breast tumours. With no toxicity observed galectin-3C is a potential agent for cancer treatment. (John et al., 2003)

With a wide range of function of galectins and their deep involvement in cancer progression, galectins became an interesting novel target for cancer treatment with the potential to inhibit tumour growth, angiogenesis, invasion, and metastasis.

6 Conclusion

Since the discovery of galectins, a big progress has been made in understanding their functions in healthy and cancer cells. It has been shown that ectopic expression or abnormal levels of galectins in the specific tissue can cause a number of pathogenicities, among these not only cancer cell invasiveness but also other processes that contribute to tumour growth and formation of metastasis.

The exact part galectins play in some pathways is yet not clear, but due to the awareness that galectins can be a major cancer target, many studies direct their attention towards them. Even though several inhibitors, partially or completely blocking the effects of galectins, have already been developed many studies are still focused on this topic.

In this thesis, I have summarized the known mechanisms, through which galectins modulate cancer cell invasiveness and therefore, promote the formation of metastasis.

7 References

Reviews are indicated by Italic font

Ahmed, R. A., Shebl, A. M., & Habashy, H. O. (2017). Expression levels of β-catenin and galectin-3 in meningioma and their effect on brain invasion and recurrence : a tissue microarray study. https://doi.org/10.20892/j.issn.2095-3941.2017.0024

Akahani, S., Nangia-Makker, P., Inohara, H., Kim, H. R. C., & Raz, A. (1997). Galectin-3: A novel antiapoptotic molecule with A functional BH1 (NWGR) domain of Bcl-2 family. *Cancer Research*, *57*(23), 5272–5276. https://doi.org/10.1158/0008-5472.can-04-1816

Apte, M. V., Pirola, R. C., & Wilson, J. S. (2012). Pancreatic stellate cells: A starring role in normal and diseased pancreas. Frontiers in Physiology, 3 AUG(August), 1–14. https://doi.org/10.3389/fphys.2012.00344

Bacigalupo, M. L., Manzi, M., Espelt, M. V., Gentilini, L. D., Compagno, D., Laderach, D. J., … Troncoso, M. F. (2015). Galectin-1 Triggers Epithelial-Mesenchymal Transition in Human Hepatocellular Carcinoma Cells. *Journal of Cellular Physiology*, *230*(6), 1298–1309. https://doi.org/10.1002/jcp.24865

Baek, S. H., Ko, J. H., Lee, J. H., Kim, C., Lee, H., Nam, D., … Ahn, K. S. (2017). Ginkgolic Acid Inhibits Invasion and Migration and TGF-β-Induced EMT of Lung Cancer Cells Through PI3K/Akt/mTOR Inactivation. *Journal of Cellular Physiology*, *232*(2), 346–354. https://doi.org/10.1002/jcp.25426

Barondes, S. H., Cooper, D. N. W., Gitt, M. A., & Leffler, H. (1994). Galectins. Structure and function of a large family of animal lectins. Journal of Biological Chemistry, 269(33), 20807–20810. https://doi.org/10.1016/j.celrep.2015.02.012

Bawumia, S., Barboni, E. A. M., Menon, R. P., & Hughes, R. C. (2003). Specificity of interactions of galectin-3 with Chrp, a cysteine- and histidine-rich cytoplasmic protein. *Biochimie*, *85*(1–2), 189–194. https://doi.org/10.1016/S0300-9084(03)00007-5

Bessard, A., Frémin, C., Ezan, F., Coutant, A., & Baffet, G. (2006). MEK/ERK-Dependent uPAR Expression is Required for Motility via Phosphorylation of P70S6K in Human Hepatocarcinoma Cells. *Journal of Cellular Physiology*, *207*(1), 12–22. https://doi.org/10.1002/JCP

Bidon-Wagner, N., & Le Pennec, J. P. (2002). Human galectin-8 isoforms and cancer. Glycoconjugate Journal, 19(7–9), 557–563. https://doi.org/10.1023/B:GLYC.0000014086.38343.98

Bojic, Ž., & Vic, L. (2014). Galectin-1 binds mucin in human trophoblast, 541–553. https://doi.org/10.1007/s00418-014-1229-7

Bornstein, P., & Sage, E. H. (2002). Matricellular proteins : extracellular modulators of cell function, 608–616.

Brabletz, T., Jung, A., Dag, S., Hlubek, F., & Kirchner, T. (1999). Beta-Catenin Regulates the Expression of the Matrix Metalloproteinase-7 in Human Colorectal Cancer. *The American Journal of Pathology*, *155*(4), 1033–1038. https://doi.org/10.1016/S0002-9440(10)65204-2

Bronsert, P., Enderle-Ammour, K., Bader, M., Timme, S., Kuehs, M., Csanadi, A., … Wellner, U. F. (2014). Cancer cell invasion and EMT marker expression: a three-dimensional study of the human cancer-host interface. *The Journal of Pathology*, *234*(3), 410–422. https://doi.org/10.1002/path.4416

Brûle, F. Van Den, Califice, S., Garnier, F., Fernandez, P. L., Berchuck, A., & Castronovo, V. (2003). Galectin-1 Accumulation in the Ovary Carcinoma Peritumoral Stroma Is Induced by Ovary Carcinoma Cells and Affects Both Cancer Cell Proliferation and Adhesion to Laminin-1 and Fibronectin, *83*(3), 377–387. https://doi.org/10.1097/01.LAB.0000059949.01480.40

Camby, I., Belot, N., Lefranc, F., Sadeghi, N., de Launoit, Y., Kaltner, H., … Kiss, R. (2002). Galectin-1 modulates human glioblastoma cell migration into the brain through modifications to the actin cytoskeleton and levels of expression of small GTPases. *Journal of Neuropathology and Experimental Neurology*, *61*(7), 585–596.

Camby, I., Belot, N., Lefranc, F., Sadeghi, N., De Launoit, Y., Kaltner, H., … Kiss, R. (2002). Galectin-1 modulates human glioblastoma cell migration into the brain through modifications to the actin cytoskeleton and levels of expression of small GTPases. *Journal of Neuropathology and Experimental Neurology*, *61*(7), 585–596. https://doi.org/10.1093/jnen/61.7.585

Camby, I., Belot, N., Rorive, S., Lefranc, F., Maurage, C. A., Lahm, H., … Kiss, R. (2001). Galectins are differentially expressed in supratentorial pilocytic astrocytomas, astrocytomas, anaplastic astrocytomas and glioblastomas, and significantly modulate tumor astrocyte migration. *Brain Pathology (Zurich, Switzerland)*, *11*(1), 12–26. https://doi.org/10.1111/j.1750-3639.2001.tb00377.x

Camby, I., Le Mercier, M., Lefranc, F., & Kiss, R. (2006). Galectin-1: A small protein with major functions. Glycobiology, 16(11). https://doi.org/10.1093/glycob/cwl025

Cao, Z. Q., & Guo, X. L. (2016). The role of galectin-4 in physiology and diseases. Protein and Cell, 7(5), 314–324. https://doi.org/10.1007/s13238-016-0262-9

Chang, L., & Karin, M. (2001). Mammalian MAP kinase signalling cascades.

Chen, D., & Che, G. (2014). Value of caveolin-1 in cancer progression and prognosis: Emphasis on cancer-associated fibroblasts, human cancer cells and mechanism of caveolin-1 expression

(Review). Oncology Letters, 8(4), 1409–1421. https://doi.org/10.3892/ol.2014.2385

Chen, H.-Y., Fermin, A., Vardhana, S., Weng, I.-C., Lo, K. F. R., Chang, E.-Y., … Liu, F.-T. (2009). Galectin-3 negatively regulates TCR-mediated CD4+ T-cell activation at the immunological synapse. *Proceedings of the National Academy of Sciences*, *106*(34), 14496–14501. https://doi.org/10.1073/pnas.0903497106

Chen, J. S., Wang, Q., Fu, X. H., Huang, X. H., Chen, X. L., Cao, L. Q., … Zhang, L. J. (2009). Involvement of PI3K/PTEN/AKT/mTOR pathway in invasion and metastasis in hepatocellular carcinoma: Association with MMP-9. *Hepatology Research*, *39*(2), 177–186. https://doi.org/10.1111/j.1872-034X.2008.00449.x

Chen, L., Yao, Y., Sun, L., & Tang, J. (2017). Galectin-1 promotes tumor progression via NF-κB signaling pathway in epithelial ovarian cancer. *Journal of Cancer*, *8*(18), 3733–3741. https://doi.org/10.7150/jca.20814

Chiang, W. F., Liu, S. Y., Fang, L. Y., Lin, C. N., Wu, M. H., Chen, Y. C., … Jin, Y. T. (2008). Overexpression of galectin-1 at the tumor invasion front is associated with poor prognosis in early-stage oral squamous cell carcinoma. *Oral Oncology*, *44*(4), 325–334. https://doi.org/10.1016/j.oraloncology.2007.03.004

Chiariotti, L., Salvatore, P., Frunzio, R., & Bruni, C. B. (2002). Galectin genes: Regulation of expression. Glycoconjugate Journal. https://doi.org/10.1023/B:GLYC.0000014073.23096.3a

Chong, Y., Tang, D., Gao, J., Jiang, X., Xu, C., Xiong, Q., … Wang, D. (2016). Galectin-1 induces invasion and the epithelial-mesenchymal transition in human gastric cancer cells via non-canonical activation of the hedgehog signaling pathway. *Oncotarget*, *7*(50), 83611–83626. https://doi.org/10.18632/oncotarget.13201

Clark, A. G., & Vignjevic, D. M. (2015). Modes of cancer cell invasion and the role of the microenvironment. Current Opinion in Cell Biology, 36, 13–22. https://doi.org/10.1016/j.ceb.2015.06.004

Cousin, J. M., & Cloninger, M. J. (2016). The Role of Galectin-1 in Cancer Progression , and Synthetic Multivalent Systems for the Study of Galectin-1. https://doi.org/10.3390/ijms17091566

Dagher, S. F., Wang, J. L., & Patterson, R. J. (1995). Identification of galectin-3 as a factor in pre-mRNA splicing. *Proceedings of the National Academy of Sciences of the United States of America*, *92*(4), 1213–1217. https://doi.org/10.1073/pnas.92.4.1213

Dan, H. C., Cooper, M. J., Cogswell, P. C., Duncan, J. A., Ting, J. P.-Y., & Baldwin, A. S. (2008). Regulation of NF-kappaB is controlled by mTOR and Raptor in association with IKK. *Genes Dev.*, *22*, 1490–

1500. https://doi.org/10.1101/gad.1662308.with

Dass, K., Ahmad, A., Azmi, A. S., Sarkar, S. H., & Sarkar, F. H. (2008). Evolving role of uPA/uPAR system in human cancers. Cancer Treatment Reviews, 34(2), 122–136. https://doi.org/10.1016/j.ctrv.2007.10.005

Debelle, L., & Tamburro, A. M. (1999). Elastin : molecular description and function, 31.

Debray, C., Vereecken, P., Belot, N., Teillard, P., Brion, J. P., Pandolfo, M., & Pochet, R. (2004). Multifaceted role of galectin-3 on human glioblastoma cell motility. *Biochemical and Biophysical Research Communications*, *325*(4), 1393–1398. https://doi.org/10.1016/j.bbrc.2004.10.181

Downward, J. (2003). Targeting RAS signalling pathways in cancer therapy. Nature Reviews Cancer, 3(1), 11–22. https://doi.org/10.1038/nrc969

Dumic, J., Dabelic, S., & Flögel, M. (2006). Galectin-3: An open-ended story. Biochimica et Biophysica Acta - General Subjects, 1760(4), 616–635. https://doi.org/10.1016/j.bbagen.2005.12.020

Ebrahim, A. H., Alalawi, Z., Mirandola, L., Rakhshanda, R., Dahlbeck, S., Nguyen, D., … Chiriva-Internati, M. (2014). Galectins in cancer: carcinogenesis, diagnosis and therapy. Annals of Translational Medicine, 2(9), 88. https://doi.org/10.3978/j.issn.2305-5839.2014.09.12

Elola, M. T., Wolfenstain-Todel, C., Troncoso, M. F., Vasta, G. R., & Rabinovich, G. A. (2007). Review Galectins : matricellular glycan-binding proteins linking cell adhesion , migration , and survival, 64, 1679–1700. https://doi.org/10.1007/s00018-007-7044-8

Espín-Palazón, R., & Traver, D. (2016). The NF-κB family: Key players during embryonic development and HSC emergence. Experimental Hematology, 44(7), 519–527. https://doi.org/10.1016/j.exphem.2016.03.010

Friedl, P., Locker, J., Sahai, E., & Segall, J. E. (2012). Classifying collective cancer cell invasion - ncb2548.pdf, 14(8), 777–784.

Friedl, P., & Wolf, K. (2003). Tumour-cell invasion and migration: Diversity and escape mechanisms. Nature Reviews Cancer, 3(5), 362–374. https://doi.org/10.1038/nrc1075

Goetz, J. G., Joshi, B., Lajoie, P., Strugnell, S. S., Scudamore, T., Kojic, L. D., & Nabi, I. R. (2008). Concerted regulation of focal adhesion dynamics by galectin-3 and tyrosine-phosphorylated caveolin-1. *Journal of Cell Biology*, *180*(6), 1261–1275. https://doi.org/10.1083/jcb.200709019

Goletz, S., Hanisch, F. G., & Karsten, U. (1997). Novel alphaGalNAc containing glycans on cytokeratins are recognized invitro by galectins with type II carbohydrate recognition domains. *Journal of Cell Science*, *110 (Pt 1*, 1585–1596.

Guha, P., Kaptan, E., Bandyopadhyaya, G., Kaczanowska, S., Davila, E., Thompson, K., … Ahmed, H. (2013). Cod glycopeptide with picomolar affinity to galectin-3 suppresses T-cell apoptosis and prostate cancer metastasis. *Proceedings of the National Academy of Sciences*, *110*(13), 5052–5057. https://doi.org/10.1073/pnas.1202653110

Hashimoto, Y., Skacel, M., & Adams, J. C. (2005). Roles of fascin in human carcinoma motility and signaling: Prospects for a novel biomarker? International Journal of Biochemistry and Cell Biology, 37(9), 1787–1804. https://doi.org/10.1016/j.biocel.2005.05.004

Hirashima, M., Kashio, Y., Nishi, N., Yamauchi, A., Imaizumi, T., Kageshita, T., … Nakamura, T. (2004). Galectin-9 in physiological and pathological conditions. Glycoconjugate Journal, 19(7–9), 593–600. https://doi.org/10.1023/B:GLYC.0000014090.63206.2f

Hittelet, A., Legendre, H., Nagy, N., Bronckart, Y., Pector, J. C., Salmon, I., … Camby, I. (2003). Upregulation of galectins-1 and -3 in human colon cancer and their role in regulating cell migration. *International Journal of Cancer*, *103*(3), 370–379. https://doi.org/10.1002/ijc.10843

Hood, J. D., & Cheresh, D. A. (2002). ROLE OF INTEGRINS IN CELL INVASION AND MIGRATION. Nature Reviews Cancer, 2(2), 91–100. https://doi.org/10.1038/nrc727

Hsu, Y., Wu, C., & Hung, J. (2013). Galectin-1 promotes lung cancer tumor metastasis by potentiating integrin α 6 β 4 and Notch1 / Jagged2 signaling pathway, *34*(6), 1370–1381. https://doi.org/10.1093/carcin/bgt040

Ilmer, M., Mazurek, N., Gilcrease, M. Z., Byrd, J. C., Woodward, W. A., Buchholz, T. A., … Bresalier, R. S. (2016). Low expression of galectin-3 is associated with poor survival in node-positive breast cancers and mesenchymal phenotype in breast cancer stem cells. *Breast Cancer Research*, *18*(1), 1–12. https://doi.org/10.1186/s13058-016-0757-6

Inohara, H., Akahani, S., Koths, K., & Raz, A. (1996). Interactions between Galectin-3 and Mac-2-Binding, (313), 4530–4535.

John, C. M., Leffler, H., Kahl-knutsson, B., John, C. M., Leffler, H., Kahl-knutsson, B., … Jarvis, G. A. (2003). Truncated Galectin-3 Inhibits Tumor Growth and Metastasis in Orthotopic Nude Mouse Model of Human Breast Cancer Truncated Galectin-3 Inhibits Tumor Growth and Metastasis in Orthotopic Nude Mouse Model of Human Breast Cancer 1, *9*(June), 2374–2383.

Katoh, Y., & Katoh, M. (2008). Hedgehog signaling , epithelial-to-mesenchymal transition and miRNA (Review), 271–275. https://doi.org/10.3892/ijmm

Kim, H.-J., Do, I.-G., Jeon, H.-K., Cho, Y. J., Park, Y. A., Choi, J.-J., … Bae, D.-S. (2013). Galectin 1 expression is associated with tumor invasion and metastasis in stage IB to IIA cervical cancer.

Human Pathology, *44*(1), 62–68. https://doi.org/10.1016/j.humpath.2012.04.010

Kim, S. J., Choi, I. J., Cheong, T. C., Lee, S. J., Lotan, R., Park, S. H., & Chun, K. H. (2010). Galectin-3 Increases Gastric Cancer Cell Motility by Up-regulating Fascin-1 Expression. *Gastroenterology*, *138*(3), 1035–1045.e2. https://doi.org/10.1053/j.gastro.2009.09.061

Kim, S. J., Shin, J. Y., Lee, K. D., Bae, Y. K., Choi, I. J., Park, S. H., & Chun, K. H. (2011). Galectin-3 facilitates cell motility in gastric cancer by Up-regulating protease-activated receptor-1(PAR-1) and matrix metalloproteinase-1(MMP-1). *PLoS ONE*, *6*(9). https://doi.org/10.1371/journal.pone.0025103

Kobayashi, T., Shimura, T., Yajima, T., Kubo, N., Araki, K., Tsutsumi, S., … Raz, A. (2011). Transient gene silencing of galectin-3 suppresses pancreatic cancer cell migration and invasion through degradation of β-catenin. *International Journal of Cancer*, *129*(12), 2775–2786. https://doi.org/10.1002/ijc.25946

Kolundz, N., & Vic, L. (2011). Galectin-1 Is Part of Human Trophoblast Invasion Machinery - A Functional Study In Vitro, *6*(12). https://doi.org/10.1371/journal.pone.0028514

Krześlak, A., & Lipińska, A. (2004). Galectin-3 as a multifunctional protein. Cellular & Molecular Biology Letters, 9(2), 305–328. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/15213811

Li, S., & Li, Q. (2014). Cancer stem cells and tumor metastasis (review). International Journal of Oncology, 45(6), 1806–1812. https://doi.org/10.3892/ijo.2014.2362

Li, X., Deng, W., Nail, C. D., Bailey, S. K., Kraus, M. H., Ruppert, J. M., & Lobo-ruppert, S. M. (2006). Snail induction is an early response to Gli1 that determines the efficiency of epithelial transformation, 609–621. https://doi.org/10.1038/sj.onc.1209077

Lin, J., Lin, W., Ye, Y., Wang, L., Chen, X., Zang, S., & Huang, A. (2017). Kindlin-2 promotes hepatocellular carcinoma invasion and metastasis by increasing Wnt/β-catenin signaling. *Journal of Experimental and Clinical Cancer Research*, *36*(1), 1–14. https://doi.org/10.1186/s13046-017-0603-4

Liu, F. T., & Rabinovich, G. A. (2005). Galectins as modulators of tumour progression. Nature Reviews Cancer, 5(1), 29–41. https://doi.org/10.1038/nrc1527

Liu, W., Hsu, D. K., Chen, H. Y., Yang, R. Y., Carraway, K. L., Isseroff, R. R., & Liu, F. T. (2012). Galectin-3 regulates intracellular trafficking of EGFR through alix and promotes keratinocyte migration. *Journal of Investigative Dermatology*, *132*(12), 2828–2837. https://doi.org/10.1038/jid.2012.211

López-Lucendo, M. F., Solís, D., André, S., Hirabayashi, J., Kasai, K. I., Kaltner, H., … Romero, A. (2004). Growth-regulatory human galectin-1: Crystallographic characterisation of the structural changes

induced by single-site mutations and their impact on the thermodynamics of ligand binding. *Journal of Molecular Biology*, *343*(4), 957–970. https://doi.org/10.1016/j.jmb.2004.08.078

Margadant, C., Van Den Bout, I., Van Boxtel, A. L., Thijssen, V. L., & Sonnenberg, A. (2012). Epigenetic regulation of galectin-3 expression by β1 integrins promotes cell adhesion and migration. *Journal of Biological Chemistry*, *287*(53), 44684–44693. https://doi.org/10.1074/jbc.M112.426445

Matarrese, P., Fusco, O., Tinari, N., Natoli, C., Liu, F. T., Semeraro, M. L., … Iacobelli, S. (2000). Galectin-3 overexpression protects from apoptosis by improving cell adhesion properties. *International Journal of Cancer*, *85*(4), 545–554. https://doi.org/10.1002/(SICI)1097-0215(20000215)85:4<545::AID-IJC17>3.0.CO;2-N

Matsumura, F. (2005). Regulation of myosin II during cytokinesis in higher eukaryotes. Trends in Cell Biology, 15(7), 371–377. https://doi.org/10.1016/j.tcb.2005.05.004

Matsumura, F., Totsukawa, G., Yamakita, Y., & Yamashiro, S. (2001). Role of myosin light chain phosphorylation in the regulation of cytokinesis. Cell Structure and Function, 26(6), 639–644. https://doi.org/10.1247/csf.26.639

Mattila, P. K., & Lappalainen, P. (2008). Filopodia : molecular architecture and cellular functions, 9(june). https://doi.org/10.1038/nrm2406

Maurer, L. M., Ma, W., & Mosher, D. F. (2015). Dynamic structure of plasma fibronectin. Critical Reviews in Biochemistry and Molecular Biology, 51(4), 213–227. https://doi.org/10.1080/10409238.2016.1184224

Meng, F., Joshi, B., & Nabi, I. R. (2015). Galectin-3 overrides PTRF/cavin-1 reduction of PC3 prostate cancer cell migration. *PLoS ONE*, *10*(5), 1–15. https://doi.org/10.1371/journal.pone.0126056

Mercurio, A. M., & Rabinovitz, I. (2001). Towards a mechanistic understanding of tumor invasion - Lessons from the α6β4 integrin. Seminars in Cancer Biology, 11(2), 129–141. https://doi.org/10.1006/scbi.2000.0364

Mercurio, A. M., Rabinovitz, I., & Shaw, L. M. (2001). The alpha 6 beta 4 integrin and epithelial cell migration. Current Opinion in Cell Biology, 13(5), 541–545. https://doi.org/10.1016/S0955-0674(00)00249-0

Mierke, C. T., Frey, B., Fellner, M., Herrmann, M., & Fabry, B. (2011). Integrin α5β1 facilitates cancer cell invasion through enhanced contractile forces. *Journal of Cell Science*, *124*(3), 369–383. https://doi.org/10.1242/jcs.071985

Mirandola, L., Yu, Y., Cannon, M. J., Jenkins, M. R., Rahman, R. L., Nguyen, D. D., … Chiriva-Internati, M.

(2014). Galectin-3 inhibition suppresses drug resistance, motility, invasion and angiogenic potential in ovarian cancer. *Gynecologic Oncology*, *135*(3), 573–579. https://doi.org/10.1016/j.ygyno.2014.09.021

Nabeshima, K., Inoue, T., Shimao, Y., & Sameshima, T. (2002). Matrix metalloproteinases in tumor invasion: role for cell migration. Pathol Int, 52(4), 255–264. https://doi.org/1343 [pii]

Nangia-Makker, P., Hogan, V., & Honjo, Y. (2002). Inhibition of human cancer cell growth and metastasis in nude mice by oral intake of modified citrus pectin. *The National Cancer …*, *94*(24). Retrieved from http://jnci.oxfordjournals.org/content/94/24/1854.short

Neoh, C.-A., Wu, W.-T., Dai, G.-F., Su, J.-H., Liu, C.-I., Su, T.-R., & Wu, Y.-J. (2017). Flaccidoxide-13-Acetate Extracted from the Soft Coral Cladiella kashmani Reduces Human Bladder Cancer Cell Migration and Invasion through Reducing Activation of the FAK/PI3K/AKT/mTOR Signaling Pathway. *Molecules*, *23*(2), 58. https://doi.org/10.3390/molecules23010058

O'Driscoll, L., Linehan, R., Liang, Y. H., Joyce, H., Oglesby, I., & Clynes, M. (2002). Galectin-3 expression alters adhesion, motility and invasion in a lung cell line (DLKP), in vitro. *Anticancer Research*.

Ochieng, J., Leite-Browning, M. L., & Warfield, P. (1998). Regulation of cellular adhesion to extracellular matrix proteins by galectin-3. *Biochemical and Biophysical Research Communications*, *246*(3), 788–791. https://doi.org/10.1006/bbrc.1998.8708

Ochieng, J., Warfield, P., Green-Jarvis, B., & Fentie, I. (1999). Galectin-3 regulates the adhesive interaction between breast carcinoma cells and elastin. *Journal of Cellular Biochemistry*, *75*(3), 505–514. https://doi.org/10.1002/(SICI)1097-4644(19991201)75:3<505::AID-JCB14>3.0.CO;2-I

Ohta, H., Aoyagi, K., Fukaya, M., Danjoh, I., Ohta, A., Isohata, N., … Sasaki, H. (2009). Cross talk between hedgehog and epithelial-mesenchymal transition pathways in gastric pit cells and in diffuse-type gastric cancers. *British Journal of Cancer*, *100*(2), 389–398. https://doi.org/10.1038/sj.bjc.6604846

Ose, R., & Nagase, T. (2012). Galectin-1 and Galectin-3 Mediate Protocadherin-24-Dependent Membrane Localization of β -catenin in Colon Cancer Cell Line HCT116, 18–26.

Owens, L. V, Xu, L., Craven, R. J., Tumors, H., Xu, L., Craven, R. J., … Liu, E. T. (1995). Overexpression of the Focal Adhesion Kinase (p125 FAK) in Invasive Human Tumors. *Cancer Research*, *55*(13), 2752–2755.

Pai, S. G., Carneiro, B. A., Mota, J. M., Costa, R., Leite, C. A., Barroso-Sousa, R., … Giles, F. J. (2017). Wnt/beta-catenin pathway: Modulating anticancer immune response. Journal of Hematology and Oncology, 10(1), 1–13. https://doi.org/10.1186/s13045-017-0471-6

Paňková, K., Rösel, D., Novotný, M., & Brábek, J. (2010). The molecular mechanisms of transition between mesenchymal and amoeboid invasiveness in tumor cells. Cellular and Molecular Life Sciences, 67(1), 63–71. https://doi.org/10.1007/s00018-009-0132-1

Park, J. W., Voss, P. G., Grabski, S., Wang, J. L., & Patterson, R. J. (2001). Association of galectin-1 and galectin-3 with Gemin4 in complexes containing the SMN protein, *27*(17), 3595–3603.

Park, M. H., Ahn, B.-H., Hong, Y.-K., & Min, D. S. (2009). Overexpression of phospholipase D enhances matrix metalloproteinase-2 expression and glioma cell invasion via protein kinase C and protein kinase A/NF-kappaB/Sp1-mediated signaling pathways. *Carcinogenesis*, *30*(2), 356–365. https://doi.org/10.1093/carcin/bgn287

Park, G. Bin, Kim, D., Kim, Y., Lee, H., Kim, C. W., & Hur, D. Y. (2015). Silencing of galectin-3 represses osteosarcoma cell migration and invasion through inhibition of FAK / Src / Lyn activation and β -catenin expression and increases susceptibility to chemotherapeutic agents, (15), 185–194. https://doi.org/10.3892/ijo.2014.2721

Pastuszak, M., Groszewski, K., Pastuszak, M., Dyrla, P., Wojtuń, S., & Gil, J. (2015). Cytokeratins in gastroenterology. Systematic review. Przeglad Gastroenterologiczny, 10(2), 61–70. https://doi.org/10.5114/pg.2015.51182

Paz, A., Haklai, R., Elad-Sfadia, G., Ballan, E., & Kloog, Y. (2001). Galectin-1 binds oncogenic H-Ras to mediate Ras membrane anchorage and cell transformation. *Oncogene*, *20*(51), 7486–7493. https://doi.org/10.1038/sj.onc.1204950

Peng, L., Liu, Z., Xiao, J., Tu, Y., Wan, Z., Xiong, H., … Xiao, W. (2017). MicroRNA-148a suppresses epithelial-mesenchymal transition and invasion of pancreatic cancer cells by targeting Wnt10b and inhibiting the Wnt/-catenin signaling pathway. *Oncology Reports*, *38*(1), 301–308. https://doi.org/10.3892/or.2017.5705

Qin, Y., Rodin, S., Simonson, O. E., & Hollande, F. (2017). Laminins and cancer stem cells: Partners in crime? Seminars in Cancer Biology, 45, 3–12. https://doi.org/10.1016/j.semcancer.2016.07.004

Rabinovitz, I., & Mercurio, A. M. (1998). Cell migration on laminin-1 by mediating the motility structures.

Rosenbergs, I., Cherayils, J., & Isselbacher, J. (1991). Mac-2-binding Glycoproteins.

Saussez, S., & Kiss, R. (2006). Galectin-7. Cellular and Molecular Life Sciences, 63(6), 686–697. https://doi.org/10.1007/s00018-005-5458-8

Schaffner, F., Ray, A. M., & Dontenwill, M. (2013). Integrin α5β1, the fibronectin receptor, as a pertinent

therapeutic target in solid tumors. Cancers, 5(1), 27–47. https://doi.org/10.3390/cancers5010027

Schaller, M. D., Hildebrand, J. D., Shannon, J. D., Fox, J. W., Vines, R. R., & Parsons, J. T. (1994). Autophosphorylation of the focal adhesion kinase, pp125FAK, directs SH2-dependent binding of pp60src. *Molecular and Cellular Biology*, *14*(3), 1680–1688. https://doi.org/10.1128/MCB.14.3.1680.Updated

Seetharaman, J., Kanigsberg, A., Slaaby, R., Leffler, H., Barondes, S. H., & Rini, J. M. (1998). X-ray crystal structure of the human galectin-3 carbohydrate recognition domain at 2.1-A resolution. *The Journal of Biological Chemistry*, *273*(21), 13047–13052. https://doi.org/10.1074/jbc.273.21.13047

Serini, G., Trusolino, L., Saggiorato, E., Cremona, O., De Rossi, M., Angeli, A., … Marchisio, P. C. (1996). Changes in integrin and E-cadherin expression in neoplastic versus normal thyroid tissue. *Journal of the National Cancer Institute*, *88*(7), 442–449. https://doi.org/10.1093/jnci/88.7.442

Serizawa, N., Tian, J., Fukada, H., Baghy, K., Scott, F., Chen, X., … Jiang, J. X. (2015). Galectin 3 regulates HCC cell invasion by RhoA and MLCK activation. *Laboratory Investigation*, *95*(10), 1145–1156. https://doi.org/10.1038/labinvest.2015.77

Shalom-Feuerstein, R., Cooks, T., Raz, A., & Kloog, Y. (2005). Galectin-3 regulates a molecular switch from N-Ras to K-Ras usage in human breast carcinoma cells. *Cancer Research*, *65*(16), 7292–7300. https://doi.org/10.1158/0008-5472.CAN-05-0775

Shen, K., Li, C., Chien, L., Huang, C., Su, C., Liao, A. C., & Wu, T. (2016). Role of galectin-1 in urinary bladder urothelial carcinoma cell invasion through the JNK pathway. https://doi.org/10.1111/cas.13016

Shimura, T., Takenaka, Y., Tsutsumi, S., Hogan, V., Kikuchi, A., & Raz, A. (2004). Galectin-3, a novel binding partner of beta-catenin. *Cancer Research*, *64*(18), 6363–6367. https://doi.org/10.1158/0008-5472.CAN-04-1816

Shukla, S., MacLennan, G. T., Hartman, D. J., Fu, P., Resnick, M. I., & Gupta, S. (2007). Activation of PI3K-Akt signaling pathway promotes prostate cancer cell invasion. *International Journal of Cancer*, *121*(7), 1424–1432. https://doi.org/10.1002/ijc.22862

Smith, H. W., & Marshall, C. J. (2010). Regulation of cell signalling by uPAR. Nature Reviews Molecular Cell Biology, 11(1), 23–36. https://doi.org/10.1038/nrm2821

Song, S., Ji, B., Ramachandran, V., Wang, H., Hafley, M., Logsdon, C., & Bresalier, R. S. (2012). Overexpressed galectin-3 in pancreatic cancer induces cell proliferation and invasion by binding

ras and activating ras signaling. *PLoS ONE*, *7*(8). https://doi.org/10.1371/journal.pone.0042699

Song, S., Mazurek, N., Liu, C., Sun, Y., Ding, Q. Q., Liu, K., … Bresalier, R. S. (2009). Galectin-3 Mediates Nuclear β-Catenin Accumulation and Wnt Signaling in Human Colon Cancer Cells by Regulation of GSK-3β Activity, *69*(4), 1343–1349. https://doi.org/10.1158/0008-5472.CAN-08-4153.Galectin-3

Sung, W., Kim, H., & Park, K.-K. (2016). The biological role of epithelial-mesenchymal transition in lung cancer (Review). Oncology Reports, 1199–1206. https://doi.org/10.3892/or.2016.4964

Tai, Y.-L., Chen, L.-C., & Shen, T.-L. (2015). Emerging roles of focal adhesion kinase in cancer. BioMed Research International, 2015, 690690. https://doi.org/10.1155/2015/690690

Takenaka, Y., Fukumori, T., & Raz, A. (2002). Galectin-3 and metastasis. Glycoconjugate Journal, 19(7–9), 543–549. https://doi.org/10.1023/B:GLYC.0000014084.01324.15

Tang, D., Zhang, J., Yuan, Z., Gao, J., Wang, S., Ye, N., … Jiang, K. (2014). Pancreatic satellite cells derived galectin-1 increase the progression and less survival of pancreatic ductal adenocarcinoma. *PLoS ONE*, *9*(3), 1–13. https://doi.org/10.1371/journal.pone.0090476

Tang, D., Zhang, J., Yuan, Z., Zhang, H., Chong, Y., Huang, Y., … Wang, D. (2017). PSC-derived Galectin-1 inducing epithelial-mesenchymal transition of pancreatic ductal adenocarcinoma cells by activating the NF-κB pathway. *Oncotarget*, *8*(49), 86488–86502. https://doi.org/10.18632/oncotarget.21212

Tang, X., Zha, L., Li, H., Liao, G., Huang, Z., Peng, X., & Wang, Z. (2017). Upregulation of GNL3 expression promotes colon cancer cell proliferation, migration, invasion and epithelial-mesenchymal transition via the Wnt/β-catenin signaling pathway. *Oncology Reports*, *38*(4), 2023–2032. https://doi.org/10.3892/or.2017.5923

Tanjore, H., & Kalluri, R. (2006). The Role of Type IV Collagen and Basement Membranes in Cancer Progression and Metastasis. The American Journal of Pathology, 168(3), 715–717. https://doi.org/10.2353/ajpath.2006.051321

Teperino, R., Aberger, F., Esterbauer, H., Riobo, N., & Pospisilik, J. A. (2014). Canonical and non-canonical Hedgehog signalling and the control of metabolism. Seminars in Cell & Developmental Biology, 33, 81–92. https://doi.org/10.1016/j.semcdb.2014.05.007

Tian, H., Zhou, Y., Yang, G., Geng, Y., Wu, S., Hu, Y., … Wu, W. (2016). Sulforaphane-cysteine suppresses invasion via downregulation of galectin-1 in human prostate cancer DU145 and PC3 cells. *Oncology Reports*, *36*(3), 1361–1368. https://doi.org/10.3892/or.2016.4942

Tinari, N., Kuwabara, I., Huflejt, M. E., Shen, P. F., Iacobelli, S., & Liu, F. T. (2001). Glycoprotein 90K/MAC-2BP interacts with galectin-1 and mediates galectin-1-induced cell aggregation. *International Journal of Cancer. Journal International Du Cancer*, *91*(2), 167–172. https://doi.org/10.1002/1097-0215(20010115)91:2<167::AID-IJC1022>3.0.CO;2-Z [pii]

Tousant III, L. G. T., Nilson, A. E., Goble, J. M., Ballman, K. V, James, C. D., Lefranc, F., … Uhm, J. H. (2012). Galectin-1 , a gene preferentially expressed at the tumor margin , promotes glioblastoma cell invasion, 1–13.

Vervloessem, T., Kerkhofs, M., La Rovere, R. M., Sneyers, F., Parys, J. B., & Bultynck, G. (2017). Bcl-2 inhibitors as anti-cancer therapeutics: The impact of and on calcium signaling. Cell Calcium, 70, 102–116. https://doi.org/10.1016/j.ceca.2017.05.014

Vignjevic, D., Schoumacher, M., Gavert, N., Janssen, K. P., Jih, G., Laé, M., … Robine, S. (2007). Fascin, a novel target of β-catenin-TCF signaling, is expressed at the invasive front of human colon cancer. *Cancer Research*, *67*(14), 6844–6853. https://doi.org/10.1158/0008-5472.CAN-07-0929

Wang, Y. G., Kim, S. J., Baek, J. H., Lee, H. W., Jeong, S. Y., & Chun, K. H. (2012). Galectin-3 increases the motility of mouse melanoma cells by regulating matrix metalloproteinase-1 expression. *Experimental and Molecular Medicine*, *44*(6), 387–393. https://doi.org/10.3858/emm.2012.44.6.044

White, K. L., Rider, D. N., Kalli, K. R., Knutson, K. L., Jarvik, G. P., & Goode, E. L. (2011). Genomics of the NF-κB signaling pathway: Hypothesized role in ovarian cancer. *Cancer Causes and Control*, *22*(5), 785–801. https://doi.org/10.1007/s10552-011-9745-4

Wieczorek, E., Wasowicz, W., Gromadzinska, J., & Reszka, E. (2014). Functional polymorphisms in the matrix metalloproteinase genes and their association with bladder cancer risk and recurrence : A mini-review, 744–752. https://doi.org/10.1111/iju.12431

Wojtukiewicz, M. Z., Hempel, D., Sierko, E., Tucker, S. C., & Honn, K. V. (2015). Protease-activated receptors (PARs)—biology and role in cancer invasion and metastasis. Cancer and Metastasis Reviews, 34(4), 775–796. https://doi.org/10.1007/s10555-015-9599-4

Wu, K. L., Huang, E. Y., Jhu, E. W., Huang, Y. H., Su, W. H., Chuang, P. C., & Yang, K. D. (2013). Overexpression of galectin-3 enhances migration of colon cancer cells related to activation of the K-Ras-Raf-Erk1/2 pathway. *Journal of Gastroenterology*, *48*(3), 350–359. https://doi.org/10.1007/s00535-012-0663-3

Wu, M.-H., Hong, T.-M., Cheng, H.-W., Pan, S.-H., Liang, Y.-R., Hong, H.-C., … Chen, Y.-L. (2009). Galectin-1-Mediated Tumor Invasion and Metastasis, Up-Regulated Matrix Metalloproteinase

Expression, and Reorganized Actin Cytoskeletons. *Molecular Cancer Research*, *7*(3), 311–318. https://doi.org/10.1158/1541-7786.MCR-08-0297

Yamazaki, D., Kurisu, S., & Takenawa, T. (2005). Regulation of cancer cell motility through actin reorganization. Cancer Science, 96(7), 379–386. https://doi.org/10.1111/j.1349-7006.2005.00062.x

Yang, R. Y., Hsu, D. K., & Liu, F. T. (1996). Expression of galectin-3 modulates T-cell growth and apoptosis. *Proceedings of the National Academy of Sciences of the United States of America*, *93*(13), 6737–6742. https://doi.org/10.1073/pnas.93.13.6737

Yoshioka, K., Nakamori, S., & Itoh, K. (1999). Overexpression of Small GTP-binding Protein RhoA Promotes Invasion of, (13), 2004–2010.

Zhang, D., Chen, Z., Liu, S., Dong, Z., Dalin, M., Bao, S., … Wei, F. (2013). Galectin-3 gene silencing inhibits migration and invasion of human tongue cancer cells in vitro via downregulating β-catenin. *Acta Pharmacologica Sinica*, *34*(1), 176–184. https://doi.org/10.1038/aps.2012.150

Zheng, D., Hu, Z., He, F., Gao, C., Xu, L., Zou, H., … Wang, J. (2014). Downregulation of galectin-3 causes a decrease in uPAR levels and inhibits the proliferation, migration and invasion of hepatocellular carcinoma cells. *Oncology Reports*, *32*(1), 411–418. https://doi.org/10.3892/or.2014.3170

Zheng, J., Lu, W., Wang, C., Xing, Y., Chen, X., & Ai, Z. (2017). Galectin-3 induced by hypoxia promotes cell migration in thyroid cancer cells. *Oncotarget*, *8*(60), 101475–101488. https://doi.org/10.18632/oncotarget.21135

Zhu, X., Wang, K., Zhang, K., Xu, F., Yin, Y., Zhu, L., & Zhou, F. (2016). Galectin-1 knockdown in carcinoma-associated fibroblasts inhibits migration and invasion of human MDA-MB-231 breast cancer cells by modulating MMP-9 expression, *48*(March), 462–467. https://doi.org/10.1093/abbs/gmw019

Zou, X. Z., Liu, T., Gong, Z. C., Hu, C. P., & Zhang, Z. (2017). MicroRNAs-mediated epithelial-mesenchymal transition in fibrotic diseases. European Journal of Pharmacology, 796(110), 190–206. https://doi.org/10.1016/j.ejphar.2016.12.003