From Department of Biosciences and Nutrition
Karolinska Institutet, Huddinge, Sweden

PAK4 IN ORGAN DEVELOPMENT AND CANCER INITIATION

Parisa Rabieifar

Karolinska Institutet

Stockholm 2021

Published by Karolinska Institutet.
Printed by Universitetsservice US-AB, 2021

ISBN 978-91-8016-253-1
Cover illustration: Pancreatic intraepithelial neoplastic (PanIN) lesion from 6 months old female *Pdx-Cre; Kras* ^{G12D}*; PAK4* $^{fl/fl}$ mouse.

PAK4 IN ORGAN DEVELOPMENT AND CANCER INITIATION
THESIS FOR DOCTORLA DEGREE (Ph.D.)

By

Parisa Rabieifar

The thesis will be defended in public at DNA (5105), Hälsovägen 7 C, Neo building, Karolinska Institutet, Huddinge
18th June 2021, 1:00pm

Principal Supervisor:
Professor Staffan Strömblad
Karolinska Institutet
Department of Biosciences and Nutrition

Co-supervisor:
Professor Klas Wiman
Karolinska Institutet
Department of Oncology-Pathology

Opponent:
Professor Ruth Palmer
University of Gothenburg
Department of Medical Biochemistry and Cell Biology

Examination Board:
Associate Professor Stephan Teglund
Karolinska Institutet
Department of Comparative Medicine

Professor Maréne Landström
Umeå University
Department of Medical Biosciences

Professor Margaret Sällberg Chen
Karolinska Institutet
Department of Dental Medicine

ABSTRACT

Tumors are complex organs with a unique ecosystem containing tumor cells entangled with various infiltrating cells. Tumors have distinct signaling signatures, which confers upon it the ability to grow in the primary host organ and further disseminate to other parts of the body. Tumors are a heterogeneous mix of sub-clones raised through genetic evolution. Concurrently, strong evidence suggests that nongenetic variables such as developmental cues add to this functional heterogeneity within individual tumors. Interestingly, multiple pathways involved in human organ development are restored and upregulated in various adult cancer.

p21-activated kinase 4 (PAK4) is a downstream effector of the Cdc42 and Rac1 small Rho family GTPases. PAK4 is involved in embryonic development, but its expression is also upregulated in different cancer types. Formerly, reverse genetic efforts to study PAK4 have been hampered due to the embryonic lethality under the complete depletion of PAK4. Consequently, multiple conditional *Pak4* knockout murine models have been developed to study the possible role of PAK4 in various stages of tissue development.

The overall aim of this thesis is to explore the role of PAK4 in breast and pancreas organ development and to dissect its role in the formation of pancreatic ductal adenocarcinoma (PDAC).

In **paper I**, we dissected the role of PAK4 in mammary gland development. Conditional *Pak4* gene depletion in the murine mammary gland did not affect this organ's normal physiology or development. Moreover, *Pak4* depletion was dispensable for normal murine pancreas development and whole-body hemostasis maintenance **paper II**. Therefore, the mouse model developed in **paper II** was further crossed with the *Pdx-Cre; K-ras*$^{G12D/+}$ model to investigate the role of *Pak4* in PDAC formation in **paper III**.

We demonstrate in **paper III** that *Pak4* depletion significantly reduces the formation of pre-neoplastic lesions via inhibition of Kras^{G12D}-driven acinar to ductal reprogramming (ADR). The aforementioned halt is accompanied by increased senescence-like growth arrest and decreased apoptosis.

Notably, *PAK4* gene expression was higher in human PDAC tumors than the normal tissue, and its protein expression was elevated in human pancreatic intraepithelial neoplasia (PanIN) and PDAC compared with the normal tissues.

In sum, this thesis improves our understanding of the role of PAK4 in organ development and provides insight into the possible role of PAK4 in PDAC initiation and progression.

LIST OF SCIENTIFIC PAPERS

I. **Parisa Rabieifar**, Ting Zhuang, Tânia Costa, Miao Zhao and Staffan Strömblad (2019). Normal mammary gland development after MMTV-Cre mediated conditional PAK4 gene depletion. *Scientific Reports* 9, 14436. https://doi.org/10.1038/s41598-019-50819-4
*Parisa Rabieifar and Ting Zhuang contributed equally.

II. Miao Zhao, **Parisa Rabieifar**, Tânia Costa, Ting Zhuang, Audrey Minden, Matthias Löhr, Rainer Heuchel and Staffan Strömblad (2017). Pdx1-Cre-driven conditional gene depletion suggests PAK4 as dispensable for mouse pancreas development. *Scientific Reports* 7, 7031. https://doi.org/10.1038/s41598-017-07322-5

III. **Parisa Rabieifar**, Miao Zhao, Carlos. F. Moro, Tânia D.F. Costa, Bela Bozoky, Nicholas P Tobin, Matthias Löhr, Rainer Heuchel, Daniel Öhlund, Staffan Strömblad. PAK4 governs Kras-driven premalignant acinar cell reprogramming. *Manuscript*.

Scientific paper not included in the thesis

I. Tânia Costa, Ting Zhuang, Julie Lorent, Emilia Turco, Helene Olofsson, Miriam Masia-Balague, Miao Zhao, **Parisa Rabieifar**, Neil Robertson, Raoul Kuiper, Jonas Sjölund, Matthias Spiess, Pablo Hernández-Varas, Uta Rabenhorst, Pernilla Roswall, Ran Ma, Xiaowei Gong, Johan Hartman, Kristian Pietras, Peter D. Adams, Paola Defilippi and Staffan Strömblad (2019). PAK4 suppresses RELB to prevent senescence-like growth arrest in breast cancer. *Nature Communications* 10, 3589. https://doi.org/10.1038/s41467-019-11510-4

CONTENTS

LIST OF ABBREVIATIONS

ACR	Acinar cell reprogramming
ADM	Acinar to ductal metaplasia
AID	Autoinhibitory domain
ALDH	Aldehyde dehydrogenase
CA 19-9	Carbohydrate antigen 19-9
CA 125	Carbohydrate antigen 125
CACs	Centroacinar cells
CEA	Carcinoembryonic antigen
CK19	Cytokeratin 19
E	Embryonic day
ECM	Extracellular matrix
EMT	Epithelial-mesenchymal transition
ES	Embryonic stem cell
GPCR	G protein-coupled receptor
GEMM	Genetically engineered mouse model
GTT	Glucose tolerance test
IPMN	Intraductal papillary mucinous neoplasm
ITT	Insulin tolerance test
KC	PDX1-Cre/Lox-Stop-Lox (LSL)-$Kras^{G12D}$
LMNB1	Nuclear lamina protein B1
MB	Mammary bud
MCN	Mucin-producing neoplasm
MMP	Matrix metalloproteinase
MMTV-LTR	Mouse mammary virus long terminal repeat
MPCs	Multipotent progenitor cells
MT	Metallothionein
PAKs	p21-activated kinases
PAK4	p21-activated kinase 4
PanIN	Pancreatic intraepithelial neoplasia
PBD	p21-binding domain

PDAC	Pancreatic ductal adenocarcinoma
PDX	Patient-derived xenograft
Pdx1	Pancreatic and duodenum homeobox protein 1
PEN	Pancreatic endocrine neoplasm
PI3Ks	Phosphoinositide 3-Kinases
Prl	Prolactin
PrlR	Prolactin receptor
Pr	Progesterone
Ptf1a	Pancreas associated transcription factor 1a
PTHrP	Parathyroid hormone-related protein
SA-β-Gal	Senescence-associated beta-galactosidase
SAHF	Senescent associated heterochromatin foci
SASP	Senescence-associated secretory phenotype
Shh	Sonic hedgehog
Sox9	SYR-Box transcription Factor 9
STAT	Signal transducer and activator of transcription
TDLUs	Terminal duct lobular units
TEBs	Terminal end buds
TIF	Telomere dysfunction-induced foci
TME	Tumor microenvironment
WAP	Whey acidic protein
YAP	Yes-associated protein

1 INTRODUCTION

1.1 Organ development

Cells are the smallest structural units of living organisms (*1*). During development, distinct stem cells undergo a differentiation hierarchy and evolve to become progenitor cells, and ultimately differentiate into various cell types in a series of cell divisions (*2-4*). Under the influence of internal and external signaling, differentiated cells undergo continuous proliferation and migration that govern the subsequent tissue and organ development (*5-7*).

1.2 The mammary gland

1.2.1 Development, organization, and function

Feeding the newborn with nutritious milk secreted from a cutaneous gland (i.e., mammary gland) is the unique aspect of mammalian physiology, which caused the naming of the class Mammalia by Carl Linnaeus (*8*). Despite the diverse anatomical characteristics by which mammals can be identified, lactation is considered the dominant character, reflecting its importance during early stages of mammalian development (*9*).

Mammary glands are epidermal, exocrine glands that evolved more than 300 million years ago from apocrine sweat glands, and their primary function is lactation (*10, 11*). Upon birth, mammary gland represent an embryonic rudiment of the gland, which undergoes extensive expansion during puberty in response to hormonal changes. Throughout the female lifetime, the mammary gland encounters tremendous structural and functional changes induced by the menstrual cycle, pregnancy, lactation, and involution (*12*). Mice have five pairs of mammary glands located within the mammary fat pads, while in humans, there is only one pair of mammary glands enclosed in breasts (*13*). Mammary gland development consists of three distinct stages: embryonic, pubertal, and reproductive.

Embryonic mammary gland development

In mice, mammary gland embryonic development take place between embryonic day (E) 10.5 and E18.5 (*14, 15*). Between day E10 and E11, the ectoderm enlarges to form the milk line extended from anterior to posterior limbs. At E11.5, five pairs of ectodermal placodes emerge along the milk line, rising slightly above the ectoderm (*14, 16*). Around E12.5, the placode sinks into the dermis and forms buds surrounded by mammary mesenchymal cells (*17*). Between E15.5 and E16, each bud proliferates and gives rise to sprouts that invade the mammary fat precursor (*18*). In female, the sprouts will eventually form a lumen that invaginates the epidermis and shape the nipple **[Figure 1]**. A significant difference between the mouse mammary gland and human breast at birth is that in mice, the rudimentary gland is formed from a single network of mammary branches, originated from the nipple, while in humans, several little ductal trees are joined at the nipple (*19*). Moreover, in male mouse embryos, activation of androgen receptors between E13.5 and E15.5 triggers mammary bud

degradation (*20*). This is in contrast to human mammary gland embryonic development, in which both male and female glands develop similarly during embryogenesis (*19*).

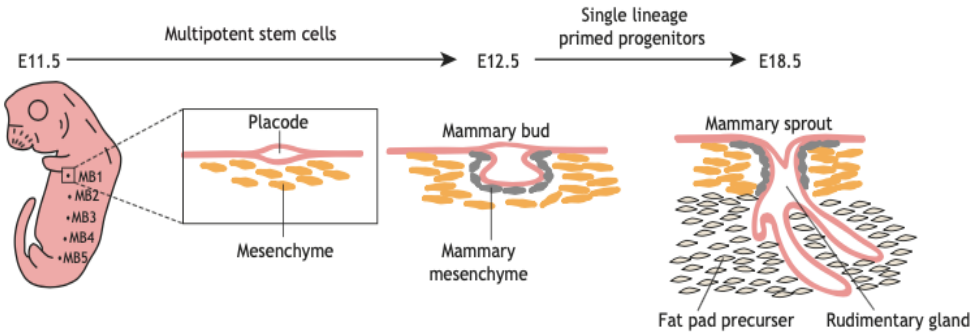

Figure 1: Schematic summary of embryonic mammary gland development.

Five pairs of mammary placodes are visible at E11.5. Placodes later invaginate to the dermis below them and form mammary buds which are in proximity to the mammary mesenchyme. Between E15 and E18.5, each bud proliferates, and form sprouts that eventually give rise to primary embryonic mammary branches. MB: mammary bud.

From: (*17*)- Copyright: Permission from Professor Christine J. Watson

Pubertal mammary gland development

The mammary gland remains unchanged from birth to puberty (*21*). During puberty, hormonal release from the pituitary gland and ovaries orchestrates the formation of terminal end buds (TEBs), a spherical structure that encapsulates the tip of the primary ducts. Upon estrogen (E) exposure, TEBs generate additional primary ducts, leading to expansion of lateral secondary branching **[Figure 2.A]** (*22*). Ductal growth further continues until the mammary tree reaches the end of the mammary fat pad (5 weeks in mice and 18-24 years in humans) (*23, 24*). Once the mammary gland reaches its maximum length, TEBs stop proliferating and shrink in size (*25*). The final tree-like structure consists of luminal epithelial cells, which are surrounded by contractile myoepithelium. The epithelium proliferates and diminishes during each menstrual cycle (*26*). In mice, the fully developed mammary gland is filled with adipocytes, while in humans, the mammary stroma is enriched in fibrous connective tissue (*27*).

Reproductive mammary gland development

A crucial difference between mouse and human mammary glands is the timing of the lobular unit formation. In the human terminal duct lobular units (TDLUs) are already formed during puberty, whereas, in mice, the formation of lobuloalveolar structures only appears at the onset of the pregnancy and exposure to an excess of progesterone (*28, 29*). In the late stages of pregnancy and lactation, and under the influence of prolactin, luminal epithelial cells produce milk and secret it into the alveoli of the lumen. Further, the suckling of the nipple by the newborn stimulates oxytocin secretion. In response to oxytocin surrounding myoepithelium contract, and thus facilitates the moving of the milk through the ducts and into the nipple. Upon

weaning and lack of milk suckling of the newborn, the ductal structure goes through apoptosis and shrinkage to regains its inherent size in an involution event (*30*).

1.2.2. Signaling pathways in mammary gland development

The embryonic development of the mammary gland depends on the corresponding signaling between epithelium and the mesenchyme, which is regulated by a myriad of factors, e.g., estrogen, androgen, transcription factors, growth factors, extracellular matrix (ECM) components, as well as epithelial-mesenchymal signaling through parathyroid hormone-related peptide (PTHrP) (*31-34*).

In the mouse embryo, the early embryonic placodes are surrounded by a layer of primary mesenchymal cells that regulates cell proliferation and invasion of the bud's tip that eventually leads to the formation of primary sprouts (*35*). Continued signaling from primary mesenchymal cells contributes to the formation of a ductal tree at birth. After birth, matrix metalloproteinases (MMP) induce ECM remodeling during lateral branching of the mammary gland and facilitate further expansion of the mammary tree (*36, 37*).

Once pregnancy occurs, progesterone (Pg) and prolactin (Prl) induce extensive side-branching, differentiation, and formation of the lobuloalveolar structure required for milk secretion during lactation **[Figure 2.A]** (*38-41*). When the lobuloalveolar structure is developed, further development of secretory mammary epithelium is dependent on prolactin receptor (PrlR) and downstream signaling, where signal transducer and activator of transcription5 (Stat5) secure epithelial proliferation and differentiation of mammary alveoli during pregnancy (*38, 42*).

Upon weaning of the newborn, excess milk leaks to the mammary epithelium, initiating the involution process in which mammary epithelial cells regain their initial mammary duct features (*11*). Involution occurs in two phases: an initial reversible phase which is induced by local cues and occurs 48 hours after weaning and is characterized by massive apoptosis, alveolar cell detachment, and discharging of cells into the lumen (*43-45*). The STAT (Signal transducer and activator of transcription) family of proteins plays a crucial role during this first phase of involution, where STAT5A and STAT5B positively regulate survival signals through the PI3Ks (Phosphoinositide 3-kinases) pathway, while STAT3 acts as a negative regulator (*46, 47*). The second phase is irreversible and is regulated by secreted factors such as serine proteases and MMPs. The expression of these factors initiates during the first phase of involution; however, they remain to be activated until the beginning of the second phase. Following MMPs and proteases' activation, basal membrane remodels and alveoli collapse, which eventually takes back the mammary tree to its non-lactating state (*48, 49*).

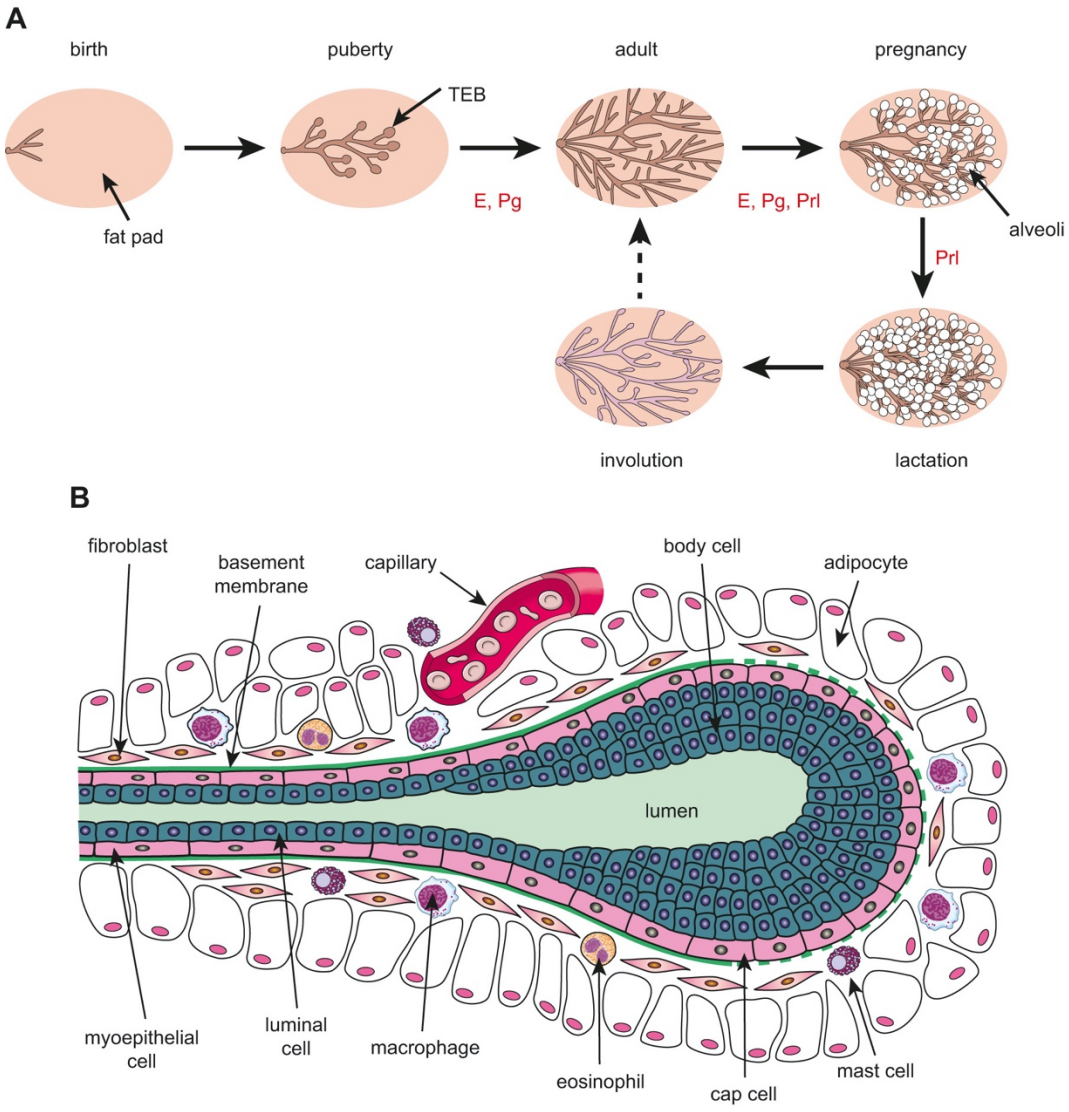

Figure 2: Schematic summary of postnatal mammary gland development in the mouse.

A: Mammary gland remains dormant until puberty. At the onset of puberty and under the influence of estrogen, ductal morphogenesis occurs. Progesterone further regulates the side branching. Once pregnancy occurs, estrogen, progesterone, and prolactin contribute to alveolar expansion. Prolactin is the main regulator of the late stage of pregnancy and lactation. E: Estrogen, Pg: Progestrone, Prl: Prolactin.
B: Structure of terminal end bud during puberty.

From: (*50*). Copyright © 2020, The American Physiological Society

1.2.3 Different cell types of the mammary gland

Epithelial cells

The mature mammary gland is compromises of apical, luminal epithelial cells expressing Keratin 8 and 18, and myoepithelial cells in contact with basal membrane cells and express Keratin 5, 14, and smooth muscle actin (*51-55*). There are multiple epithelial cells in TEBs such as: (Cap) epithelial cells that appear at the end of TEBs and are in contact with the stroma through a thin basal lamina, the body cells that are in the interior part of the end buds, and the central body cells that go through apoptosis to form the lumen **[Figure 2.B]** (*51*).

Adipocytes

Adipocytes cover a large portion of the mammary gland, contributing to epithelial growth, angiogenesis, and communicate with other cell types within the gland (*56, 57*). During pregnancy and lactation, adipocyte volume is reduced, suggesting a role in milk production (*56, 58*).

Fibroblasts

Fibroblasts regulate communication with the epithelium during ductal branching and orchestrate the mammary gland morphogenesis via regulation of the ECM composition. Moreover, fibroblasts contribute to the synthesis of various growth factors and MMPs, which degrades the ECM and releases the growth factors embedded within the ECM (*59-61*).

Vascular cells

During puberty, the lymphatic and vascular networks develop in proximity to the mammary epithelial tree to facilitate carrying nutrients and fluids into milk (*62, 63*).

Immune cells

Macrophages and eosinophils are required to regulate the invasion of branching tips through the mammary fat pad. They also regulate epithelial cell death and adipocyte reconstitution during involution (*64, 65*).

1.3. The pancreas

1.3.1. Development, organization, and function

The pancreas is an organ located in the stomach's abdominal cavity. It is about 12-25 cm in length and has 70 to 150 grams of weight in human. The head of the pancreas is connected to the duodenum, where the main pancreatic ducts are attached to the bile duct, and its narrow tail extends to the left side of the body very close to the spleen. The pancreas consists of two glandular structures: the exocrine pancreas, which contributes to food digestion by releasing enzymes into the duodenum, and the endocrine pancreas that maintains blood glucose level **[Figure 3]** (*66-68*).

Exocrine pancreas

The exocrine pancreas compartment makes up 95% of the organ, consisting of acinar, centroacinar, and ductal cells, and in mice, it develops at ~E11.5-12.5 (*66, 69*).

Adult acini are pyramid-shaped exocrine cells that contain a pronounced amount of endoplasmic reticulum and a massive number of secretory granules containing digestive enzymes that are secreted into the duodenum via the pancreatic duct **[Figure 3]**. The specification of acini is determined by pancreas-associated transcription factor 1a (Ptf1a) (*70*). During differentiation, acinar cells express Rbpjl and Mist1; the consequent mature acini express Ptf1a, Gata4, Mist1 (Bhlha15), and Nr5a2 (*71, 72*). Pancreatic acini are connected to the small pancreatic ducts via centroacinar cells (CACs) (*73*). CACs are derived from multipotent progenitor cells (MPCs), and they keep expressing some of the progenitor markers such as Ptf1a, Pdx1, Nkx6.1, and Sox9 throughout the development (*74-76*). CACs maintain their progenitor-like feature in the adult pancreas, with sustained Notch signaling that secures their identity (*77*).

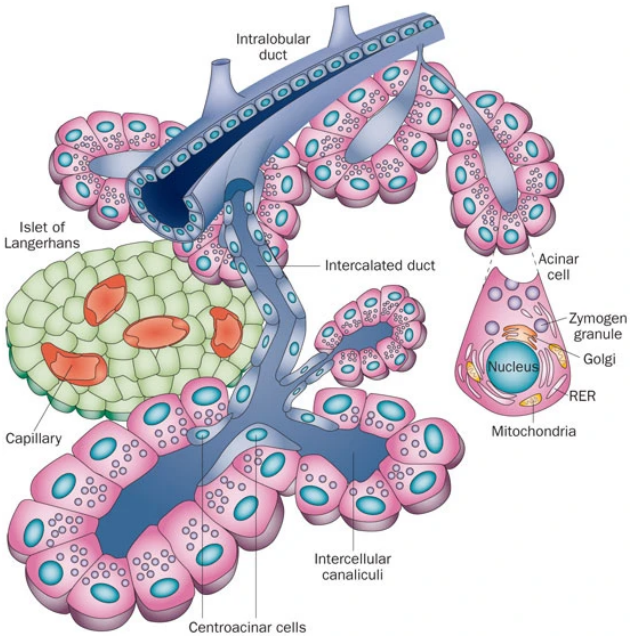

Figure 3: Schematic representation of the pancreas.

The pancreas consists of exocrine and endocrine compartments. Pancreatic acinar cells locate around a central lumen which opens to the ducts. Endocrine pancreases consist of Islets of Langerhans (pancreatic islets), formed in proximity with pancreatic ducts.

From: (*78*). Copyright © 2013 springer nature

Endocrine pancreas

The endocrine gland is formed by cell clusters called "islets of the Langerhans" or "pancreatic islets," which make up 1-2% of the organ's weight (*66*). Pancreatic islets contain five different cell types that produce endocrine hormones: 1) α-cells producing glucagon, 2) β-cells producing insulin and amylin, 3) γ-cells producing pancreatic polypeptide(pp) 4) δ-cells producing somatostatin, and 5) ε-cells producing ghrelin. These endocrine hormones work together to secure a balanced blood glucose level. Where insulin decreases the blood sugar level, glucagon increases it, and somatostatin regulates both insulin and glucagon's secretion. The islet structure is quite similar among mammals; however, there is a difference between human and rodent islets. Rodent islets are made of β-cells in the middle surrounded by peripheral α-cells while human islets are formed of interconnected α and β-cells **[Figure 4]** (*79, 80*).

Both the exocrine and endocrine pancreas are primarily composed of epithelial cells. Nevertheless, endocrine islet forming cells lose their epithelial connectivity with the lumen and tight junctions later during the development and create cord-like structures with proximity along the duct and blood vessels. Within the islets, positive glucagon cells appear at E9, while insulin-containing granules are not seen until the subsequent transition periods (*81*). After formation, β-cells slowly lose their contact with the mesenchymal layer, mainly supported by ECM molecules in the basement membrane such as fibronectin, laminin-1, collagen IV, and integrin. The location of endocrine cells and their appearance are dependent on close interactions with the ECM, especially with the basal membrane layer (*82*).

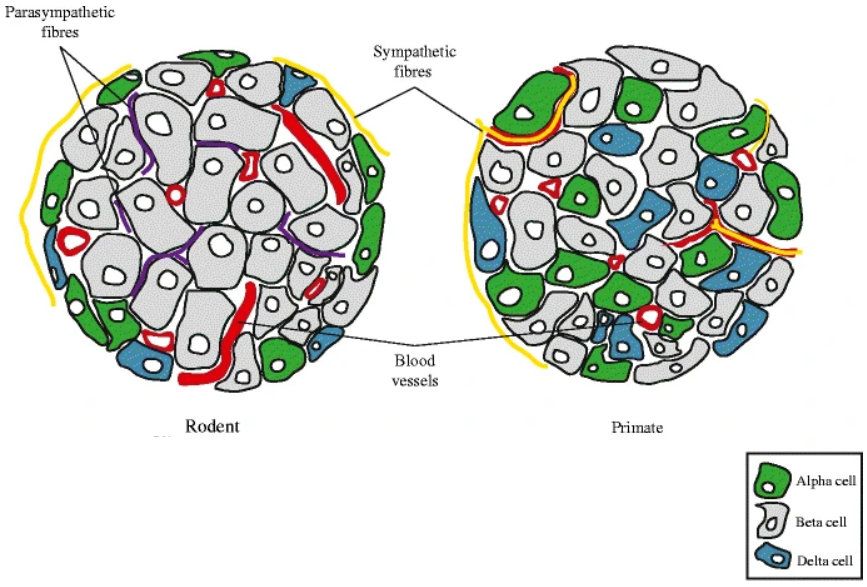

Figure 4: Schematic representation of islets of the pancreas in humans and mice.

While primate islets formed from a random distribution of cells, rodent islets have a uniform architecture with α and δ cells located on the periphery of the islets and β-cells in the center.

From: (*80*). Copyright © 2015, Springer-Verlag Berlin Heidelberg

1.3.2. Signaling pathways in pancreas development

Early pancreas development signaling

Signaling involved in early pancreas development is derived from the notochord, and the surrounding mesenchyme. Before the start of the pancreatic budding, dorsal notochord releases morphogenic signaling such as activin βB and FGF2 to suppress sonic hedgehog (*Shh*) signaling and allowing pancreatic gene expression and organogenesis (*83*). Soon after, mesenchymes release FGF10, BMPs, and follistatin which in turns activate pancreatic and duodenal homeobox 1 (*Pdx1*) at E8.5, followed by pancreas-specific transcription factor 1a (*Ptf1a*) at E9.5 (*84-86*). From E9.5 to E12.5, Notch signaling oversees maintaining multipotent progenitor cells (MPCs) pool, MPCs, in turn, co-express Ptf1a, Sox9, and Pdx1 and eventually gives rise to all types of pancreatic cells (*87-93*).

Early pancreas morphogenesis signaling

The transition of the MPCs to the complete organ is a two-step process (*94*). The primary step starts at E12.5 and contains the segregation of tip and trunk domains. It is regulated by the Notch pathway and crosstalk between mesenchyme and endothelium (*95-97*). In which tip cells are identified by expression of Cpa, c-Myc, and Ptf1a, and the trunk domain is identified by expression of Onecut-1, Tcf2, Hes1, Prox1, and Sox9 (*74-76, 98-102*). In the second phase of transition, tip cells give rise to acinar cells under the influence of Ptf1a, Rbp-jl, and Nr5a2/LRH-1, while trunk cells are bipotential and form both endocrine and ductal cells. A fraction of cells in the trunk domain express transcription factor Ngn3 and transform to endocrine cells (*102-104*). However, the subset of cells which do not activate Ngn3 gain a ductal cell phenotype and become ductal cells via expression of Sox9, Tcf2, Onecut1, Hes1, Prox1, and Glis3 **[Figure 5]** (*101, 105-111*).

Mature endocrine and exocrine development signaling

Wnt/β-catenin signaling pathway is the master regulator of acinar cell development by maintaining the MPCs pool (*112*). Furthermore, follistatin secreted from mesenchyme contributes to acinar cell differentiation (*113, 114*). Like exocrine, endocrine development is depending on the Wnt/β-catenin signaling pathway, which regulates β-cell number and ensures its complete differentiation (*115*).

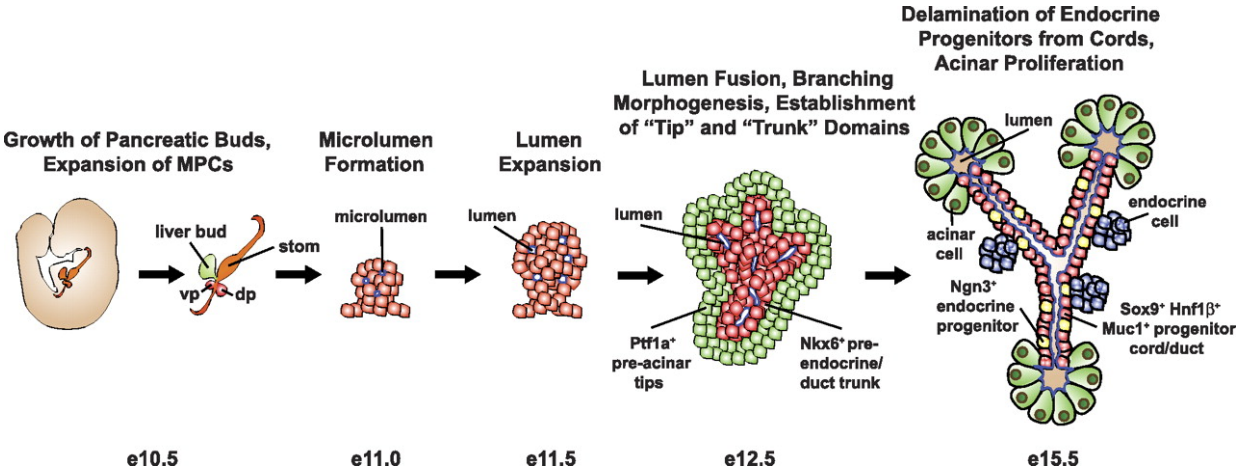

Figure 5: Early pancreas morphogenesis.

The mature pancreas is formed from pancreatic progenitor cells. Within the first phase of transition, progenitor cells maintain pancreatic cell's identity. During the second transition phase, progenitor cells obtain either tip or trunk identity. While tip cells further develop to acinar cells, trunk cells undergo further signaling to become either endocrine or ductal cells.

From: (*116*). Copy right © 2011, American Diabetes Association 2011

1.4 Cancer

Cancer existed long before human existence. Paleopathological findings indicate evidence of tumors in animals in prehistoric times. The earliest evidence of cancer in a human was first introduced by Edwin Smith Papyrus, dated approximately 3000 BC. The terms Carcinoma (malignant tumor) and Cancer (ulcerated malignant tumor) were first introduced by Hippocrates (460-375 BC). The word Onco (swelling) was later introduced by Greek physician Galen 130-200 AD (*117*).

Cancer is not just one disease but a large group of disorders, characterized by their unregulated cell growth, uncontrolled cell division, and ability to spread to other parts of the body. There are more than 200 known cancer types, of which approximately 70% are derived from epithelial cells (*118, 119*). Cancer is characterized by defective cells that are not following the "normal" rules of tissue hemostasis. The steps leading to the transformation of normal cells to become cancer cells and subsequent spread of cancer cells to metastatic regions are well described in the "Hallmarks of cancer" by Hanahan and Weinberg (*120, 121*).

1.4.1 Hallmarks of cancer

As normal cells lose their inherent features and acquire a neoplastic state, they obtain a series of traits that enables them to become tumorigenic and eventually develop the ability to spread to other organs. In 2000, Douglas Hanahan and Robert Weinberg described the six crucial "core" traits that facilitate cancer growth and their subsequent spread to the metastatic region, including 1) avoiding growth suppressors, 2) sustained proliferative signaling, 3) resistance to cell death, 4) enabling replicative immortality, 5) activating invasion and metastasis, and 6) inducing angiogenesis. A decade later, the authors updated the list of hallmarks by adding four new identifying traits. Two "emerging hallmarks", 7) evading immune response, and 8) interfering with cellular energetics, and two "consequential hallmarks", which facilitate the gaining of both "core" and "emerging hallmarks": 9) genome instability and 10) tumor-promoting inflammation (*120, 122*).

1.4.2 Cellular Senescence

Aging is a common characteristic of multicellular organisms and is defined by the inability to maintain the function of multiple cells or a particular tissue. Aging often accompanies both loss of function alterations, like what is seen in age-related degenerative diseases, and gain-of-function changes that allow the cells to propagate indefinitely, such as cancer.

Cancer is an age-related, gain-of-function disease in which cells acquire the ability to proliferate, migrate and colonize to an ectopic site via bypassing different hallmarks of cancer (*123*). Both hyperplastic and degenerative aging diseases commonly go through a stress response named "cellular senescence", which imposes a proliferation arrest on damaged cells and facilitates tissue hemostasis maintenance (*124*).

Cellular senescence occurs in response to myriads of stimuli such as DNA damage, telomere shortening, mitogens, proliferation-associated signals, mitochondrial dysfunction, epigenomic damage, and tumor suppressor activation (*123*). It was initially believed that senescence is essentially a permanent-growth arrest. However, recent studies indicate that, senescent cells can acquire a certain degree of stemness upon chemotherapy that helps them escape from cell-cycle blockade (*125*).

There is no universal marker to detect senescence cells; instead, senescent cells are defined by several characteristics (*126*). Senescent cells are characterized by being larger and more flattened than healthy cells and can be detected by the presence of senescence-associated-β-galactosidase (SA-β-gal), a marker of lysosomal degradation (*127, 128*). Other markers of senescent cells are senescence-associated secretory phenotype (SASP), presence of telomere dysfunction- induced foci (TIF), senescent associated heterochromatin foci (SAHF), increased expression of the P16INK4a tumor suppressor, and nuclear lamina protein B1 (LMNB1) (*129-131*).

1.5 Pancreatic cancer

1.5.1 Pancreatic cancer etiology

Pancreatic cancer is the 7th leading cause of cancer death, causing around 4% of all cancer deaths with only 9%, 5-years survival rate, and a median survival of <11 months (*132-134*). The term pancreatic cancer refers to tumors rising from exocrine, endocrine, and ductal cells. However, more than 90% of the pancreatic neoplasms have ductal differentiation (*135*). The remaining 10% are "non-ductal" tumors, including pancreatic endocrine neoplasm (PEN), pancretoblastoma, and solid-pseudopapillary neoplasm (*136*).

Many risk factors contribute to pancreatic cancer occurrences, such as smoking, family history of the disease, chronic pancreatitis, obesity, helicobacter infection, and periodontal disease (*137-142*). Seven to 10% of all pancreatic cancer cases are attributed to inherited mutations such as mutations in tumor suppressor *STK11*, *BRCA1* or *BRCA2*, germline mutation in *CDKN2A,* and DNA repair genes (i.e., *MLH1*, *MSH2*, *MSH6*, and *PALB2*) (*143-146*).

Pancreatic cancers' dismal prognosis is often attributed to its difficulty to be diagnosed, mainly due to its very mild, if any, symptoms before it develops to advanced stages. Unfortunately, the symptoms present at the time of diagnosis are often attributed to benign conditions and delayed diagnosis. Some of the commonly reported symptoms include abdominal pain, jaundice, new-onset diabetes, nausea, weight loss, and back pain (*147, 148*). The routine diagnostic techniques are multidetector CT angiography and MRI. Carbohydrate antigen 19-9 (CA19-9), carcinoembryonic antigen (CEA), and CA125 are used as serum biomarkers for pancreatic cancer diagnosis, with CA19-9 having the highest specificity of 82% for diagnosis in symptomatic patients (*149*).

1.5.2 Pancreatic ductal adenocarcinoma (PDAC)

PDAC has a bleak prognosis, with only 9% 5-year survival after diagnosis (*132*). The early stage of pancreatic cancer is usually asymptomatic, mainly due to the location of the tissue. Therefore, approximately 80% of PDAC patients are already at an advanced or metastatic stage at the time of diagnosis and therefore not eligible for undergoing surgery (*150*). The majority of PDAC tumors arise from the pancreas head and are often diagnosed earlier due to biliary obstruction (*151*). However, tumors in the pancreas body and tail are diagnosed later and have already spread to distant organs at diagnosis. Hence, PDAC in these locations is associated with a poorer prognosis (*152*). The most common symptoms are abdominal pain, jaundice, and weight loss (*153*). To better categorize and allocate therapy regimens for PDAC, multiple large-scale DNA sequencing and gene expression profiling have been performed in recent years. Two molecular subtypes have been identified: 1) "classical/canonical subtype" characterized by epithelial-like gene expression, and 2) "quasi-mesenchymal/basal-like subtype" carrying a worse prognosis than the classical subtype (*154-156*).

PDAC is an intractable disease due to its tumor heterogeneity and multiple cellular events that contribute to the formation of the disease. PDAC tumor heterogeneity in the same tumor and

among different patients makes it challenging to implement targeted therapies available for other cancer types, such as against EGFR in lung cancer or BRAF in melanoma (*157-159*). Moreover, multiple signaling pathways such as Kras, CDKN2A/p16, TP53, and SMAD4 are altered during the progression of PDAC, contributing to multiple resistance mechanisms developed during PDAC treatment (*160*). Finally, the dense desmoplastic PDAC tumor microenvironment (TME) is characterized by decreased vasculature, altered immune cell filtration, and hypoxia that facilitates tumor growth and eliminates drug delivery (*161*). To date, surgery is the primary treatment of pancreatic cancer followed by adjuvant therapy with gemcitabine, capecitabine, and mFOLFRINOX after completing surgical resection (*162*). However, these treatments are not sufficient for patients with late-stage disease (*163*).

1.5.3 PDAC initiation and acinar cell reprogramming

PDAC can rise from three different precursor lesions, intraductal papillary mucinous neoplasm (IPMN), pancreatic intraepithelial neoplasia (PanIN), and mucinous cystic neoplasm (MCN) (*164*). Most PDAC lesions originate from PanIN and less prevalently from IPMN and in some rare cases from MCN. It was believed that PanINs originate from the ductal cells, primarily due to morphological resemblance and expression of the ductal marker like cytokeratin 19 (CK19) (*165-167*). However, we now know that the most of the PanIN lesions originate from acinar cells (*94, 165, 166, 168*). Once acinar cells are insulted by acute damage, they lose their original phenotype and enter an intermediate, dedifferentiated duct-like metaplastic state, termed acinar to ductal reprogramming (ADR) **[Figure 6]** (*169*). Often, ADR is a transitionary state that is reverted once the acute damage is resolved. However, upon constant insult by oncogene activation or chronic pancreatitis, acinar cells lose their identity and acquire duct-like features. Such trans-differentiation is called acinar to ductal metaplasia (ADM) (*170-174*).

During ADM, acinar cells lose their capacity to express acinar genes such as *Mist1*, *CPa1*, *elastase*, and amylase and instead express ductal cell genes such as cytokeratin 19 (*CK19*), mucin1 (*Muc1*), *Pdx*, and *Sox9* (*68, 175*). Both chronic pancreatitis and oncogenic Kras can initiate ADM. However, their presence alone is inadequate to further drive carcinogenesis beyond the premalignant PanIN stage to high-grade dysplasia and PDAC. Additional gene mutations, inflammation, and wild-type Kras activation are other subsequent mechanisms that facilitate PanIN progression to PDAC (*176-181*).

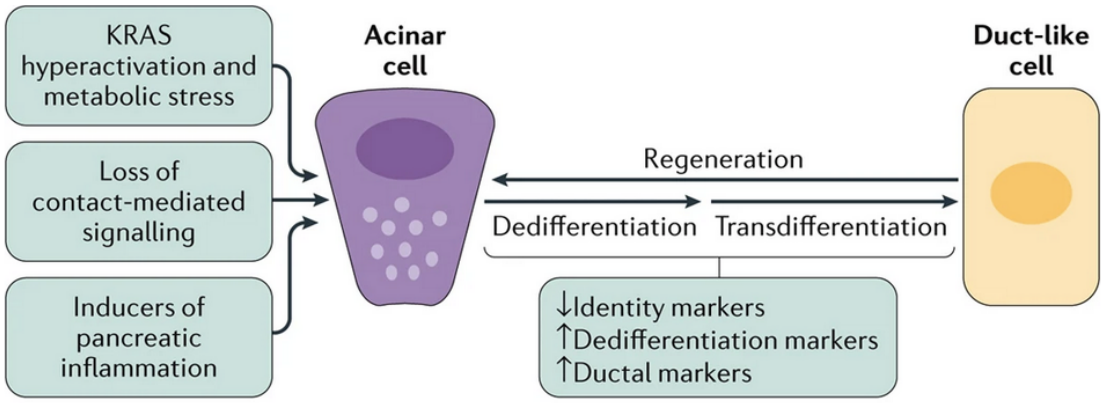

Figure 6: Acinar plasticity and PDAC development

Upon contact-mediated signaling, pancreatic injury, and oncogenic Kras activity, acinar cells undergo reversible trans-differentiation termed as acinar to ductal metaplasia (ADM), in which they lose their identity markers and obtain ductal fate markers. ADM becomes irreversible in the presence of gain of function Kras mutation.

From: (*182*). Copyright © 2017, Nature Publishing Group, a division of Macmillan Publishers Limited.

1.6. p21-activated kinases (PAKs)

The p21-activated kinases (PAKs) are serine/threonine kinases and were primarily discovered as proteins that interacted with GTP-bound Rac (*183*). PAKs act as downstream effectors of Cdc42 and Rac1 small GTPases (*183, 184*). The PAK family contains six members and is divided into two groups based on their sequence and structural homology (*185*). Group I of PAKs consists of PAK1, PAK2, and PAK3 and group II contains PAK4, PAK5 and PAK6 (*186*). The protein structure of PAKs consists of a C-terminal serine/threonine kinase domain with a phosphorylation site and an N-terminal regulatory domain. The N-terminal regulatory domain is structurally different among the two groups. Group one PAKs are regulated with an auto-inhibitory mechanism, where two PAK monomers form a homodimer through the attachment of the auto-inhibitory domain (AID) of one molecule to the other PAK molecules kinase domain. Upon binding of activated small GTPases to the p-21 binding domain (PBD), the dimerization is terminated. This leads to phosphorylation of both molecules and initiation of their further kinase activity (*187-189*). Group II PAKs have a similar structure as group I but are missing AID, and therefore they were considered to be constitutively active (*190*). However, it has been shown that the PAK4 regulatory domain contains an AID-like pseudosubstrate sequence that inactivates its kinase activity (*191, 192*).

PAKs control major cellular events such as cytoskeletal remodeling, DNA damage response, and mitotic progression. PAK family exerts their functions through their intrinsic kinase activity, their ability to shuttle between nucleus and cytoplasm, modulating the target gene expression, and employing their scaffolding activity. PAKs excessive expression is correlated

with growth signal self-sufficiency, cell proliferation, and invasion, cell survival, activating metastasis, and angiogenesis, all of which are hallmarks of cancer (*122, 193, 194*).

PAK kinases have different expression patterns throughout development. Therefore, great efforts have been made to study their functions using knockout animal models. While *Pak1*and *Pak3* knockout mouse models are viable and appear normal and fertile, *Pak2* and *Pak4* knockout mice are embryonically lethal (*195-198*).

Given PAKs' involvement in diverse cell signaling and their fundamental role in cellular hemostasis, disruption in PAK signaling can disturb normal tissue functions and lead to different types of human disorders such as cardiac and neurological disorders and different types of cancer.

1.6.1. p21-activated kinase 4 (PAK4)

PAK4 is a group II PAK and is highly expressed during development and in many adult tissues (*198, 199*). Unlike group I PAKs, PAK4 lacks an autoinhibitory domain; instead, it contains an autoinhibitory-like pseudo-substrate sequence that inhibits its kinase activity (*191*). PAK4 is involved in mediating multiple of cytoskeletal regulation such as the formation of filopodia, stress fibers disintegration, turnover of focal adhesion, as well as actin polymerization and de-polymerization (*200-202*). Also, PAK4 regulates cell adhesion via interacting with αvβ5 integrin and regulates adhesion dynamic **[Figure 7]** (*200, 203, 204*). Moreover, PAK4 excessive expression is linked to prolonged cell survival, protection of cells from apoptosis, and bypassing of the oncogene-induced-senescence (OIS) barrier (*196, 205, 206*).

Many of the cellular cues regulated by PAK4 are involved in tissue development and disturbed during cancer development; therefore, it is crucial to study the role of PAK4 in tissue development and cancer.

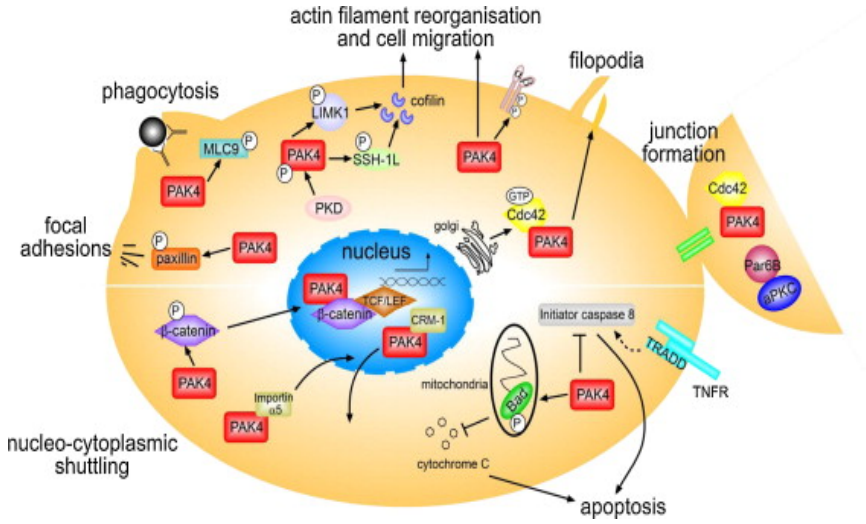

Figure 7: PAK4 regulates different cellular functions and signaling pathways

PAK4 mediates various cellular functions and signaling pathways including actin remodeling, pro-survival, anti-apoptotic pathways, and apical junction formation.

From: (*201*). Copyright © 2013 Elsevier GmbH

1.6.2. PAK4 in tissue development and cancer

PAK4 in tissue development

Embryos subjected to *Pak4* gene knockout die before E11.5 due to defects in the fetal heart, improper folding in neural tubes, and abnormalities in neuronal differentiation. Due to embryonic lethality upon total *Pak4* knockout, conditional *Pak4* gene knockout mice were developed to explore its function in development of various tissues. Although *Pak4* gene knockout in the central nervous system and heart caused a malfunction in the normal tissue function, *Pak4* conditional depletion in the mammary gland (**paper I**) and pancreas (**paper II**) did not alter either development or function of both organs (*202, 207-210*).

PAK4 in cancer

PAK4 is overexpressed in numerous cancer types such as endometrioid ovarian cancer, oral squamous cell carcinoma, and basal-like breast cancer (*190, 211-218*). In pancreatic cancer and ovarian cancer, PAK4 overexpression has been associated to PAK4 gene amplification (*213, 214, 219*). Clinically, PAK4 overexpression correlates with a poor prognosis, more aggressive phenotype, and a higher chance of metastasis and distant tissue infiltration (*220, 221*).

PAK4 promotes the transformation of normal cells to cancer cells via controlling the cell proliferation, survival, invasion, metastasis, and epithelial-mesenchymal transition (EMT) (*222-225*). Moreover, PAK4 exerts its function via interacting with diverse signaling pathways involved in cancer, such as the Wnt/β-catenin pathway, by phosphorylating and stabilizing β-catenin in the nucleus and preventing β-catenin ubiquitination (*226*). Early studies suggest that PAK4 plays a central role in actin cytoskeletal reorganization by phosphorylation of LIMK1, which subsequently phosphorylates and inactivates cofilin, reducing the ability of cofilin to depolymerize F-actin (*227, 228*). PAK4 acts as an additional target in mutant K-ras-driven cancers such as pancreatic, colorectal, and lung cancers (*229-231*). In this thesis (**paperIII**) we took a closer look at PAK4's involvement in Kras-driven pancreatic cancer development.

2 RESEARCH AIMS

Papers included in this thesis bring insight into the role of PAK4 in the development of mammary gland and pancreas, as well as in the formation of pancreatic ductal adenocarcinoma through the development and utilization of three novel transgenic mouse models.

Paper I: To set up a transgenic mouse model with conditional *Pak4* gene knockout in the mammary gland and determine the functional role of *Pak4* in mammary gland development and its function.

Paper II: To develop a novel transgenic mouse model with conditional *Pak4* gene depletion in the mouse pancreas and further assess the role of *Pak4* in pancreas development and its function.

Paper III: To define the role of *Pak4* in Kras-driven pancreatic ductal adenocarcinoma.

3 METHODOLOGICAL CONSIDERATIONS

A detailed description of the methods used in this thesis is included in each constituent paper. In this section, I will discuss the main research tools that were used in this thesis.

3.1. Animal research

Mus Musculus (house mice) have been commonly used to set up *in vivo* animal models in human research. This is due to the physiological and genetic similarities between mouse and human and the relative ease of maintenance, breeding, and gene manipulation in the mouse (*232, 233*). Gene-targeted animal models have been a practical tool to study tissue development and diseases that can be mimicked in murine cells (*234*). Traditionally, to produce offspring with certain genetic traits that resembled cancer in human, animals were exposed to different stimuli which predisposed them to human conditions, such as UV radiation and DNA-damaging chemicals (*235*).

Today, usage of homologous recombination provides opportunities to manipulate mouse embryonic stem (ES) cells and produce either genetic knockouts or knock-ins mouse models (*236, 237*). A depletory genetic alteration is a powerful tool to inactivate a gene via disruption of its DNA sequence. However, if the modified gene is vital for embryonic development, conventional knockout genetic alterations will cause embryonic lethality. Therefore, a conditional genomic alteration was developed using the Cre-lox system, in which a mutation can be induced both spatially and temporally by crossing a mouse harboring a *Lox* gene with the one carrying the target gene (*238, 239*). Given the mosaic nature of the *Cre* gene depletion system, it is crucial to detect the gene depletion on the cellular level by using immunostaining or crossing with a reporter strain (*240-242*).

PAK4 is required for embryogenesis; therefore, the Cre-lox knockout strategy has been used to generate different conditional *Pak4* knockout mouse models (*198, 207-209*). This thesis contributes to PAK4 research by introducing three novels conditional *Pak4* gene knockout mouse models. In **paper I** and **II**, we examined if *Pak4* is essential for normal mammary gland and pancreas development. In **paper III**, we studied the role of *Pak4* in a Kras-driven pancreatic ductal adenocarcinoma mouse model.

Animal models of mammary gland development

Different promoters have been used to conditionally overexpress or deplete genes from the mammary tissue using the Cre-lox system, such as mouse mammary virus long terminal repeat (MMTV-LTR), whey acidic protein (WAP), metallothionein (MT), and cytokeratin 14 (*242, 243*). Among all aforementioned promoters, Cre recombinase under the control of MMTV-LTR has been the most frequently used model to study mammary gland development due to its expression in epithelial cells and its activity during both non-lactating and lactating phases (*244*). MMTV-mediated Cre-expression is found in the mammary gland, seminal vesicles, harderian gland, and lymphoid cells (*245, 246*). Three mouse lines carrying MMTV-Cre transgene (lines A, D, and F) have been developed. It has been reported that while line D mice

can nurse their pups, both line A and F have impaired lactation, hence being unable to nurse their progeny. Therefore, it is crucial to choose the right Cre-mice line and include an MMTV-Cre control group in studies exploring mammary gland function during lactation (*247*). In **paper I**, we took advantage of MMTV-Cre (lined D) mice to study *Pak4*'s role in the mammary gland development in the juvenile, adult, and lactation phases.

Animal models of pancreas development

Several animal models have been developed by taking advantage of different transcription factors involved in pancreas development and differentiation. *Pdx1* and *Ptf1a/p48* are expressed at the early stages of pancreas development and are two main transcription factors manipulated to study pancreas development both spatially and temporally. The homeobox gene *Pdx1/IPF1* is expressed at E8.5-9 before the formation of buds, and its knocking out results in pancreas agenesis. Pdx1 expression is restricted to the pancreas, with a minor expression observed in the developing stomach and duodenum. Pdx-Cre expression controls the formation of all pancreas compartments (*91*). In **paper II**, *Pdx-Cre* promoter was used to explore the role of *Pak4* in pancreas development. PAK4 expression was below the detection limit after analyzing the pancreas lysate obtained from *Pak4* knockout mice, indicating Pdx-cre promoter expression was sufficient for successful *Pak4* gene depletion in the pancreas. However, it is crucial to consider the Cre-mosaicism previously reported on the *Pdx-1* promoter and be aware that a fraction of the pancreas epithelium will retain *Pak4* expression (*241*).

Animal models of Pancreatic ductal adenocarcinoma

Different mouse models have been employed to recapitulate the formation of PDAC *in vivo*. While early efforts involved the expression of oncoproteins and viral vectors, more recent approaches involve the usage of the Cre-lox system to create mutant mice with pancreas-specific oncogene activation (Kras) and/or inactivation of tumor suppressor genes (p53, p16INK4a, and Smad4) (*248*).

Given that Kras mutations are observed in 90% of all pancreatic adenocarcinomas, this model has been commonly used in the field (*229*). The most widely used genetically engineered mouse models are either *PDX1-Cre/Lox-Stop-Lox(LSL)-Kras*, known as KC mice, or *p48/LSL-Kras* (*249*). The benefit of using the aforementioned genetically engineered mouse models (GEMM) is that they both represent similar PDAC observed in humans by showing all stages of cancer progression from ADM and PanIN lesions to PDAC and finally metastasis to the liver, diaphragm, and pleural surfaces. Moreover, the fibroinflammatory reaction seen in both mouse models resembles those observed in human PDAC (*94, 250*). Given that both *Pdx-Cre* and *p48-Cre* mouse models mimic human PDAC and the availability of the *Pdx-Cre* mouse model in the lab (**paperII**), we used *Pdx-Cre: LSL-Kras^{G12D}* to study the role of PAK4 in PDAC *in vivo*. To develop *Pdx1-Cre; LSL-Kras ^{G12D}* mice, a targeting vector with inhibitory effect on transcription and translation is flanked by LoxP sites. The Lox-Stop-Lox (LSL) construct is inserted in *Kras* locus upstream of locus1 that harbors G-A transition in codon 12 (G12D). It is noteworthy considering that the model used in this study represents the mild

progression of PDAC in human; however, crossing these mice with lines harboring additional mutations occurring in tumor suppressor genes which are either inactivated or overexpressed in human PDAC such as *INK4A*, *TP53*, *LKB1* or *SMAD4* can speed up the progression of pancreatic lesions and give results to a full penetrance, metastasis, and formation of fully differentiated PDAC lesions as seen in human (*251*).

Despite tremendous similarities between PDAC progression in KC mice with those of human, we should note their different etiology. Firstly, KC mice are a prenatal GEMM model; however, PDAC in humans is not a pediatric disease but somewhat likely to rise due to sparse mutation in adult pancreas. Secondly, PDAC patients have Kras mutation in a small subset of their cell types, and not all pancreatic compartments as observed in KC mice. Given the phenotype observed in **paper III,** in order to further understand the mechanisms by which PDAC rise from acinar cells, it is preferable to use the second generation PDAC GEMM mouse models in which a Kras oncogene is expressed only in the acinar cells (*Elas-tTA; Tet-O-Cre; $KRas^{LSL\text{-}G12Vgeo}$*) and its expression can be controlled temporally with usage of doxycycline (*167*).

Usage of animal models advanced our understanding of both human organ development and diseases remarkably; however, species-specific differences are often neglected among researchers. Lack of recapitulation of cancer events and different responsiveness to anti-tumor regimens are among the shortcomings of using mice as the model to recapitulate human cancer. In recent years, patient-derived xenograft (PDX) and tumor-derived organoids have been used as an alternative method to GEMMs as the pre-clinical tumor models of choice for anti-cancer drug screening (*252*).

3.2. Ethical considerations

Using animals in research has been highly debatable over the years. Animals used in research might suffer from discomfort throughout the experiment; therefore, scientists are asked to state both the rationale that the knowledge acquired outweigh the suffering imposed and a detailed procedure used in their research involving animal models (*253*). In Sweden, we secure the animal model well-being by obtaining an ethical permit issued by the regional ethical committee after a thorough evaluation of an ethical application. Procedures performed in this thesis have been performed following Swedish and European Union guidelines and were approved by Stockholm south animal ethics committee. The degree of difficulty of the experimental procedures performed in animals was moderate, and we always kept the 3Rs (Replacement, Reduction, Refinement) in mind when designing animal experiments.

Using human tissue in research requires having informed consent before tissue collection. The informed concern should be under local and national regulations. Patient samples used in **paper III** of the thesis were obtained from PDAC cancer patients who underwent surgery. All the patients signed written informed consent. The study was performed according to the Helsinki Declaration of 1975 and was approved by the regional research ethics board.

4 RESULTS AND DISCUSSION

Complete gene depletion of *Pak4* in mice leads to embryonic lethality due to defects in the brain, heart, and vasculature (*207-209*). Therefore, it is crucial to investigate its role in different organ development. In **paper I** and **II**, we explored the role of *Pak4* in the mammary gland and pancreas development by developing two novel animal models with *Pak4* conditional gene knockout in the corresponding tissue. In **paper III**, we crossed the pancreas mouse model developed in **paper II** with a $Kras^{G12D}$ mouse model and investigated the role of *Pak4* in pancreatic cancer initiation and progression. In the following chapter, I will discuss the key findings obtained in each paper.

Paper I: Normal mammary gland development upon *Pak4* conditional knockout

To deplete the *Pak4* gene in the mammary gland, MMTV-Cre (line D) mice were crossed with *Pak4*-floxed mice (*207, 208, 242, 254*). The consequent *Pak4* gene knockout ($Pak4^{MEp-/-}$) and their control group ($Pak4^{MEp+/+}$) mice were born at the expected Mendelian ratio. The female and male offspring were identically distributed, indicating that *Pak4* genetic ablation does not affect the survival of either sex. We phenotypically compared the $Pak4^{MEp-/-}$ mice with their control group littermates. After performing immunolabelling, PAK4 expression was one-fourth in the mammary gland of the $Pak4^{MEp-/-}$ compared with those of $Pak4^{MEp+/+}$. We further confirmed successful *Pak4* depletion in luminal and myoepithelial cells of the mammary gland. Although a large portion of the ductal cells was negatively labeled for PAK4 in the $Pak4^{MEp-/-}$, there was still a noticeable fraction of the epithelium expressing PAK4, an indicator of incomplete Cre-penetration, as it was reported previously for MMTV-Cre-driven conditional gene depletion (*242*).

Next, the morphology of ductal structures was examined using the whole-mount of inguinal mammary glands to compare $Pak4^{MEp-/-}$ mice to their date-matched $Pak4^{MEp+/+}$. We did not observe any defect in mammary duct morphogenesis upon *Pak4* depletion in either mammary ductal elongation or branching in young (virgin week4) or mature (Virgin week 10) mammary glands.

Knowing PAK4's role in mediating both cell proliferation and invasion (*201, 218, 255*), we examined the cell proliferation status by immunofluorescent labeling of Ki67 and expression and activity of MMPs in the mammary gland tissue lysates from the virgin week 4 stage when the mammary gland is at the peak of branching. In line with our previous results, the lack of *Pak4* did not change either cell proliferation or the expression of the examined MMPs in the $Pak4^{MEp-/-}$ group.

Finally, we analyzed the ability of *Pak4* gene knockout mothers to nurse their newborns, denoted by H&E staining and carmine-filled fat pad's coverage at day 2 of lactation. We found that the mammary ducts completely covered the mammary fat pad, and the alveolar units of $Pak4^{MEp-/-}$ mice were fully developed. Moreover, the mothers lacking *Pak4* in the mammary glands could nourish their pups sufficiently, indicated by equal pup body weight upon weaning.

Our results suggest that *Pak4* inactivation in the mammary gland does not impair its development. Therefore, this model could be further used for testing *Pak4*'s role in mammary tumors and other diseases of the mammary gland.

Lack of phenotypical dysfunction in the animal developed here prompted us to use this animal model to study the role of *Pak4* in PyMT-driven mammary cancer (paper not included in this thesis) (*216*).

Paper II: Normal pancreas development upon *Pdx1-Cre* mediated *Pak4* gene depletion

To study the role of *Pak4* in pancreas development, we developed a conditional mouse model with *Pak4* gene ablation in the pancreas epithelium by crossing *Pdx1-cre* mice with *Pak4*-floxed mice (*91, 207*). Mice from *Pak4* gene knockout and control groups were born at the expected Mendelian ratio, and *Pak4* depletion did not affect any of the sexes survival. Cre-lox recombination and consequent *Pak4* depletion in the knockout mice was confirmed by both genotyping for (Cre and lox) as well as immunoblotting for PAK4 in the whole pancreas lysate.

Morphological quantification of the tissues obtained indicated no difference in the acinar, ductal, or islets of Langerhans distribution between the control and the knockout groups. Moreover, localization and ratio of different cell types within the exocrine and endocrine were not altered upon *Pak4* depletion.

Finally, *Pak4* Knockout mice showed healthy body hemostasis by maintaining the same bodyweight as their control littermates; moreover, they could maintain their blood glucose level when challenged with a glucose tolerance test (GTT). This finding resembles those of *Pak5* and *Pak6* depletion in the pancreas and suggests that each member of the group II PAKs is dispensable for pancreas development (*256, 257*). In contrast, depletion of *Pak1* or *Pak3* ablates the pancreas function to maintain whole-body glucose hemostasis (*258, 259*). This provides more evidence that different members of the PAK family have a distinct role in tissue development.

However, the current study does not address the pancreas ability to maintain whole-body glucose hemostasis upon challenges such as high-fat diet or insulin tolerance test (ITT).

Together, these data suggest that *Pak4* gene depletion does not alter mouse pancreas development and function; therefore, this animal model could be a valuable tool for testing the potential *in vivo* function of *Pak4* in pancreas diseases, including pancreatic cancer.

Paper III: *Pak4* gene ablation delays Kras-driven acinar cell reprogramming

The *PAK4* gene is frequently amplified in PDAC patient samples and overexpressed in pancreatic cancer cell lines (*193, 219*). By the knowledge acquired from **Paper II**, we took advantage of the animal model already in hand and crossed them with the established PDAC mouse model LSL; *Kras* $^{G12D/+}$, which harbors the most common Kras mutation (G12D) and recapitulates all stages of the PDAC observed in human patients (*229, 260*).

Pdx-Cre; Kras ^{G12D}*; PAK4* $^{fl/fl}$ (knockout) were obtained by crossing *Pdx1-Cre; PAK4* $^{fl/fl}$ mice with LSL; *Kras* $^{G12D/+}$ and *Pdx1-Cre; Kras* ^{G12D} (control group) by crossing *Pdx-Cre*$^{+/+}$ mice with LSL-*Kras* $^{G12D/+}$. Upon examining the littermate, *Pak4* knockout mice had a lower pancreas to body weight ratio than their control littermates, indicating less tumor burden in their pancreata upon *Pak4* ablation.

Acinar to ductal metaplasia (ADM) is the earliest anaplastic change observed in the Kras ^{G12D} model. Upon histological examination, we noticed that in the knockout mice, the area occupied by ADM was larger than controls. Moreover, the consequent metaplastic lesions formed were unable to further develop to neoplastic (PanIN) lesions, indicating an inability in the knockout mice to undergo complete acinar to ductal reprogramming. Consistently, both high-grade dysplasia and cancer lesion incidences were decreased in the *Pak4* Knockout group. This suggests that *Pak4* depletion hinders Kras-induced PDAC at the acinar to ductal reprogramming (ADR) stage. This finding concurs with the previous finding in patient PDAC samples stating that PAK4 expression peaks at early neoplastic lesions and remains high in advanced PDAC.

Further progression of ADM towards cancer is challenged by a senescence-like growth arrest (*261*). Moreover, PAK4 appears to facilitate the bypass of cellular senescence escape in breast cancer (*216*). Together with the ADM halt observed in our model, these data tempted us to explore further the possibility that *Pak4* inhibition may induce a senescence-like growth arrest in the ADM lesions, thereby delaying their further progression into PanIN lesions. Notably, *Pak4* depletion blocked cell proliferation, increased expression of a common senescence marker (p16), and inhibited apoptosis in our mouse model.

The observed ADM in our model is derived from a process called “de-differentiation,” in which mature acinar cells lose their identity and obtain more stem cell-like traits. In a healthy pancreas, this process often occurs in response to damage to the acinar cells. However, upon Kras mutation and/or inflammation, de-differentiated cells progress further to a metaplastic state and gain mature ductal cell features. The latter transition of becoming a ductal cell is called “trans-differentiation.” Once cells trans-differentiate, they gain the ability to further progress to a neoplastic state and eventually form PDAC. It has been previously reported that de-differentiation is accompanied by senescence induction and acquisition of stemness features (*261*). This, together with the finding that some de-differentiated cells in the *Pak4* depleted pancreases neither re-differentiate nor trans-differentiate to a new cell type, may suggest that

PAK4 expression might push the senescent, de-differentiated cells to further progress to PanIN lesions (*262*).

In summary, our findings establish PAK4 as a mediator of ADR during PDAC development, possibly through overcoming oncogene-induced senescence.

5 CONCLUSIONS

The results presented in this thesis demonstrate that PAK4 is dispensable for mouse mammary gland and pancreas development. Also, our finding establishes PAK4 as a promotor of Kras-driven PDAC development.

The two novel mouse models developed in the **paper I** and **II**, with conditional *Pak4* gene deletion in the mammary gland and pancreas, are valuable tools to study the role of PAK4 in normal physiology and diseases affecting these two organs. We further utilized the mouse model introduced in **paper II** to study the role of *Pak4* in Kras-induced PDAC development. Our results uncover a crucial role for PAK4 in PDAC development since *Pak4* depletion in the Kras-induced PDAC mouse model causes senescence-like growth arrest in the preneoplastic lesions **[Figure 8]**. These data, together with our recent finding that PAK4 overexpression could abrogate oncogene-induced senescence (OIS) in the mammary epithelial cells, suggests PAK4 as an important mediator that both regulates further progression of preneoplastic lesions to cancer and overcomes the OIS barrier (*216*). Given that PAK4 is amplified and overexpressed in human PDAC (**paper III**), the work presented in this thesis suggest PAK4 inhibition as a potential therapeutic strategy that could both restore preneoplastic lesions to their "un-differentiated" normal state and install a senescence-like growth arrest phenotype (*219, 263*).

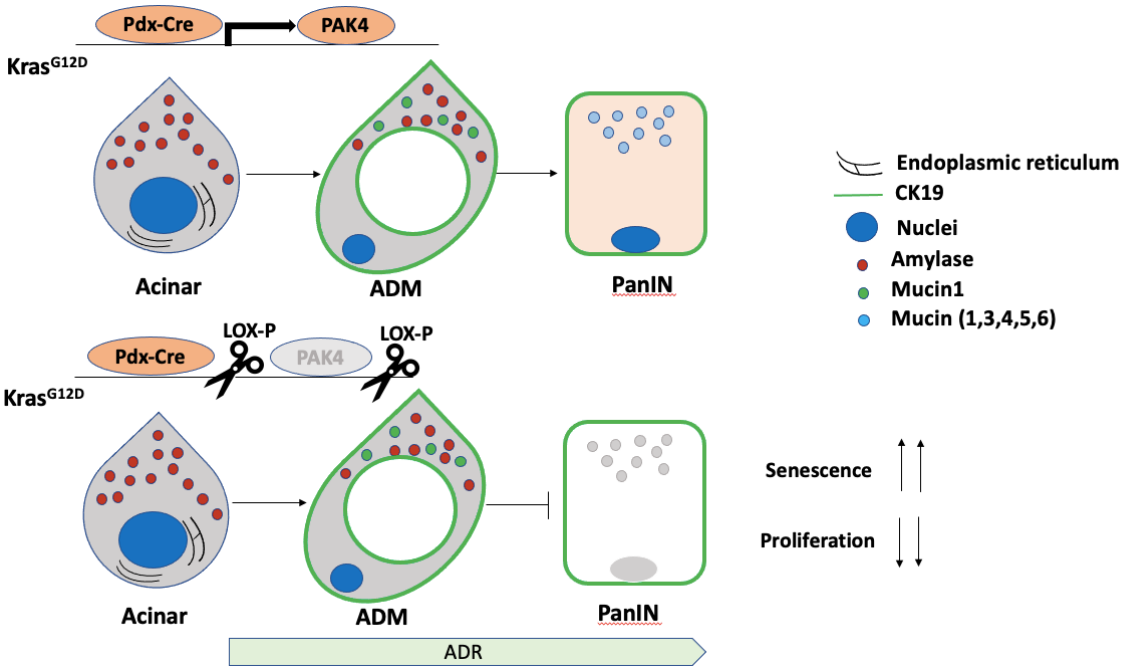

Figure 8: Schematic illustration model of the role of PAK4 in Acinar-to-Ductal reprogramming.

Upon Kras oncogene insult, pancreatic acinar cells undergo acinar-to-ductal reprogramming (ADR). PAK4 depletion remarkably suppresses ADR and PanIN formation in a mutant Kras-driven PDAC model.

6 POINTS OF PERSPECTIVE

Future research

Many confounding factors need to be determined to fully evaluate the mechanism involved in the PAK4 regulation of ADR during PDAC progression.

Both chronic and acute inflammation cooperate with oncogenic Kras to facilitate PDAC initiation and progression in humans (*167, 181, 264, 265*). Targeting inflammatory pathways in the context of PAK4 remained to be explored. This can be achieved *in vivo* by caerulein treatment, which induces excess secretion of pancreatic enzymes, and can induce either acute or chronic pancreatitis depending on the implemented dosage (*266, 267*).

PDAC tumors are resistant to therapies, partly due to their desmoplastic stromal composition formed by the excess of fibroblasts and deposition of the ECM (*268, 269*). High desmoplastic composition is linked to more poorer patients' survival. *In vivo* studies performed in $Kras^{G12D}$ mouse model (used in **paper III**) indicate that increased ECM stiffness is linked to epithelial to mesenchymal transition (EMT) and is associated with chemotherapy resistance (*270*). Therefore, it would be interesting to closely examine if there is any changes to the tumor microenvironment in terms of ECM stiffness, immune cells, and cancer-associated fibroblast composition upon *Pak4* gene depletion in our mouse model.

Clinical implications

Because PAK4 expression is increased in pancreatic lesions and PDAC tumor tissues, targeting PAK4 might be an efficient way to delay the progression of neoplastic PanIN lesions to further progress to aggressive PDAC tumors. In a clinical setting, PAK4 was directly inhibited using inhibitors such as ATP-competitive compound (PF-3758309), which unfortunately failed to pass phase I trial due to its undesirable pharmacokinetic characteristics (*271*). However, in recent years, PAK4 allosteric modulators (KPT-9274, KPR-7523, and KPT-7189) were explored, and KPT-9274 was tested in the early clinical phases (*272*). Our results suggest a role for PAK4 in the initiation stages of PDAC; this is important since PAK4 inhibition can be used as a chemo-preventive treatment to reprogram PanIN lesions back to the normal acinar cell state.

7 POPULAR SUMMARY OF THE THESIS

“Cell migration” is a process in which cells not only walk around in the tissue they belonged to but can also start to invade their neighboring tissues. Migration of cells is crucial throughout the development of the fetus and ultimately the formation of the whole body, and it continues until adulthood. It is also the most important mechanism, which helps our body to heal a wound. There are many molecules that help fine-tuning this process. One of these molecules is PAK4.

During my Ph.D. I tried to understand the role of PAK4 in the development of two organs, mammary gland (mouse milk gland), and pancreas. To do that, I worked with mice that lack the Pak4 gene in the mammary gland and pancreas (paper I &II). My results show that PAK4 is not needed for the development of these two organs.

Pancreatic cancer is an aggressive disease in which patients usually die in less than five years from the diagnosis. We know that PAK4 is expressed excessively in patients with pancreatic cancer. Therefore, I studied the role of Pak4 in the pancreatic cancer mouse model (paper III). I found out that mice lacking Pak4 in the pancreas, are less likely to develop cancer compared to the control group. My results bring hope to use of PAK4 inhibition as a therapeutic target in pancreatic cancer.

چکیده مطالب پایان نامه

مهاجرت سلولی، فرآیندی است که در آن سلولها نه تنها در بافتی که متعلق به آن هستند حرکت میکنند، بلکه می توانند به بافتهای همسایه خود نیز مهاجرت کنند. حرکت سلولی نقش بسزایی در شکل گیری جنین، تکامل اندامهای بدن وترمیم زخم ایفا میکند. سلولهای ما برای حرکت مجهز به سیستم حرکتی متشکل ازمولکولهایی هستند که زیرغشای سلولی واقع شده اند. یکی از این مولکولها پروتئینیست به نام PAK4.

من در طول دوران دکتری سعی کردم که نقش PAK4 را درشکل گیری دو اندام سینه وپانکراس (لوزالمعده) بررسی کنم. برای انجام این کار، من با موشهایی کار کردم که فاقد ژن PAK4 درغدد شیری و پانکراس بودند. نتیجه تحقیقات من نشاندهنده این است که نبود PAK4 برای رشد طبیعی این دواندام مشکلی ایجاد نمیکند (نتایج در مقاله ۱و۲).

سرطان پانکراس یکی از بدخیم ترین سرطان هاست که اغلب مبتلایان در کمتر از پنج سال بعد از تشخیص بیماری فوت میکنند. در ادامه تز دکتری نقش PAK4 را در پیشرفت سرطان پانکراس بررسی کردم (نتایج در مقاله ۳).

نتیجه تحقیقاتم نشان دهنده این است که PAK4 در بیماران سرطان پانکراس بیشتر بیان میشود وهمینطور موشهایی که این ژن را بیان نمیکنند سیر پیشرفت سرطان آهسته تری را دارند .

امید است که با در نظر گرفتن نتیجه تحقیقات من، درآینده بتوان از محدود کردن پروتئین PAK4 به عنوان یکی از اهداف درمانی سرطان پانکراس استفاده کرد

8 ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to everyone who helped and motivated me during my doctoral studies. Especially I would like to thank:

My supervisor Professor **Staffan Strömblad**, who gave me the opportunity to do a Ph.D. Your immense knowledge and great experience have always encouraged me. Thanks for your invaluable insight and for teaching me how to think independently.

My co-supervisor Professor **Klas Wiman**, for nice discussions the very few times that we met.

Professor **Maria Eriksson**, for giving me support and helpful advice throughout these years and for always being available to discuss.

Professor **Rainer Heuchel**, for sharing your knowledge in the pancreas field and for providing the mouse models used in pancreas projects. **Daniel Öhlund**, Thanks a lot for sharing your knowledge and providing valuable resources that made the continuation of the PDAC project possible. **Ankit Kumar Patel**, for introducing the organoid model. **Nick Tobin**, for the great collaboration in the pancreatic cancer project. **Carlos Fernández Moro**, thanks for the great collaboration and for always being available to answer my non-ending questions. **Raul Kuiper**, for your help in different projects and for scientific discussions.

To my dearest colleagues that made this journey possible. **Helene Oloffsson**, for being the best lab manager in the world, and for your support during these years and the positive attitude that promotes a calm and cheerful lab environment. **Feifei Yan**, for being full of life and for spontaneous chats in which we solved many world mysteries. **Sara Göransson**, for being my lab hero, I got inspired seeing your perseverance and genuine passion for science, and of course for being so cheerful after the summer breaks; expecting your smiling face always made it easier to get back to work after the breaks ☺. **Tânia Costa**, for welcoming me to the "old gang," for midsummer picnics, and after works which were all-important part of my onboarding process at SST. **Veronica Larsson**, for your extremely positive attitude towards life, for your passion for science, and for all the pep talks, whenever needed. **Xiaowei Gong**, for being a meticulous scientist, I learned a lot from your attention to detail and your persistence to do good science. **Jiangjiang Hu**, for being always available to answer my dry-lab questions and for your positive and objective attitude towards work and when we had discussions. **Jovana Gotovac,** for lovely chats during lunch breaks and scientific discussions.

To the former members of the SST lab. **Ting Zhuang** and **Miao Zhao**, for being my mentor during the first years of the Ph.D. and pursuing fundamental studies that made my thesis possible. **Miriam Masia-Balague**, for being a good friend, for after works, and for all the life obstacles that we solved through our endless discussions ☺. **Matthias Spiess**, Thanks for being a good officemate and for all the good memories throughout the years. **Uta Rabenhorst**, **Pablo Hernández-Varas**, **Ulrich Berge**, **John Lock**, **Zhilun Li**, **Xavier Serra Picamal**, **Sofie Bellamy**, **Hamdah Abbasi**, **Yuchen Cai**, **Alexa Kiss**, **Jacob Kowalawski** and **Christina**

Outskouni, thanks for all the good memories. Without you, my Ph.D. journey would not have been the same.

Gabriela Imreh, for being the most patient microscopy teacher, for always being available to chat, and simply for being a true friend throughout these years. You are a kind-hearted person that I was honored to meet during my Ph.D. studies.

Sylvie Le Guyader, for your positive attitude and always having a smile on your face and, and for caring about people around you. **Gisele Miranda**, for being so kind and easy to collaborate. **Tobias Nyberg**, for your positive attitude and chats during birthday fikas. **Monica Ahlberg**, thanks for always being approachable and being so open and pleasant to talk to. **Lars-Arne Haldosen**, for giving me the courage to admit my weaknesses and acknowledge my strength and for being a good mentor to Ph.D. students. **Maryam Saghafian**, for being a good friend throughout the years and for much needed fika sessions. **Arash Chitsazan**, thanks for always being responsive to my fika requests and for ad hoc scientific brainstorming. **Marjan abbasi**, **Yasaman Zahedi,** and **Laia Sadeghi**, thank you, girls, for all the good memories. It was lovely to have you and your positive energy around. **Farzaneh Shahin Varnoosfaderani**, for all the supports you gave me through the end of my Ph.D., for your friendship, and great company. **Joman Javadi**, For great memories. **Fahimeh Darki,** thanks for your friendship throughout the years.

Daniel Marcellino, thanks for your unwavering support and belief in me. Your passion for science always inspires me.

Mikael Allan Mikaelsson being around you quite early in my career was one of the best milestones because you strive to make the world a better place in your everyday life had impacted everyone around you, including me.

Leander Blaas and **Marco Gerling**, Thanks for valuable discussions and your positive attitude.

Ani Azatyan and **Tina Jacob**, for being kind and a reliable shoulder over the years. Thanks for being wonderful friends who made my Ph.D. life much more fun and easier to get through. **Karl annusvar**, for being so positive and always available to discuss science and the much-needed whiskey workshop. **Giorgia Palano**, thanks for being such a nice friend who was always there for me and especially for the great help throughout the final stage of my Ph.D. **Magali Merrien, Arian Eskafi**, **Ksenia Goroshchuk**, **Gonzalo Fernandez**, thank you guys for being my PhD-mates and for your friendship. It would not have been possible to go through these years without much needed after works. I wonder where life will take us after this journey, but I hope to be in touch with you wherever we ended up being. Especial thanks to **Magali Merrien**, for being a great mentor during the final stage of my Ph.D. **Anna Webb**, for the lovely fikas and for the supports you gave me during my Ph.D. **Agustin Sola Carvajal**, for organizing the much-needed DCS pubs ☺. **Ana Amaral**, **Sandra Scharaw**, **Rodrigo Lozano**, for lovely chats and a fantastic music collection that rhymed with my pipetting. **Lucia Pena Perez**, for wonderful times in the ski conference and lovely get-togethers that always gave me

positive energy. **Laia Gorchs**, **Sandra Petrus-Reurer**, for good memories at the ski conference, and the friendship lasted afterward.

To my Örebroare friends: **Yevgen Chernove,** and **Gabriela De Rossi Ayres**, thanks for wonderful friendship during the last 11 years and for years of having Fun ☺.

To my family in Sweden: **Golnaz Nozari**, thanks for being part of this journey since I came to Sweden and for always empowering me. **Afrooz Abbaspour**, for teaching me how to be alive, cheerful, and free. It's been a long ride and having you on the same journey made it easier to get through. **Mohammad Najafi**, for being a true friend during the last ten years and for being the one I can always count on. **Tommi Suvitaival**, for always being calm and positive, being around you is a blessing. **Soroor** and **Urko Serrano** for being so energetic and full of life. Seeing your endless energy makes me never give up. **Zahra Golabkesh**, **Behrooz Afsharivafa** & **Nova**, for loving, caring, and supports throughout the years. **Sahar Asadi** and **Armin Salmasi**, for being kind and for good memories through these years.

Leila Bahrami for being an inspiration throughout my life and for being the sisters that I can always count on.

پوران صفاری، پری صفاری: مرسی بابت تمام این سالها که به من عشق ورزیدن و تلاش رو یاد دادین.

مامان: تو همیشه منو تشویق کردی وبهم گفتی که من هرکاری که اراده کنم رو میتونم انجام بدم. مرسی که همیشه بال پروازم بودی .

بابا: من هروقت خسته میشدم وفکر میکردم که دیگه نمیتونم ادامه بدم یاد تو وقوی بودنت میافتادم. تو همیشه دوست داشتی که ما موفق وسربلند باشیم ولی اینو نمیدونستی که با داشتن پدری مثل تو موفق نشدنی در کار نیست .

Omid Rabieifar: I've always looked up to you, even when I was miles away from home. You were the true inspiration and the sound that reminded me that sky is the limit.

Special thanks to my partner**, Hamid Noroozi**, for your patience and supports during all these years and for the never-ending positive energy that you brought to my life.

9 REFERENCES

1. T. H. Schwann, Microscopial researches into the accordance in the structure and growth of animals and plants. 1847. *Obes Res* **1**, 408-418 (1993).
2. H. Clevers, F. M. Watt, Defining Adult Stem Cells by Function, not by Phenotype. *Annu Rev Biochem* **87**, 1015-1027 (2018).
3. H. Clevers, STEM CELLS. What is an adult stem cell? *Science* **350**, 1319-1320 (2015).
4. A. Sánchez Alvarado, S. Yamanaka, Rethinking differentiation: stem cells, regeneration, and plasticity. *Cell* **157**, 110-119 (2014).
5. D. M. Bryant, K. E. Mostov, From cells to organs: building polarized tissue. *Nat Rev Mol Cell Biol* **9**, 887-901 (2008).
6. D. Gilmour, M. Rembold, M. Leptin, From morphogen to morphogenesis and back. *Nature* **541**, 311-320 (2017).
7. L. E. O'Brien, M. M. Zegers, K. E. Mostov, Opinion: Building epithelial architecture: insights from three-dimensional culture models. *Nat Rev Mol Cell Biol* **3**, 531-537 (2002).
8. C. v. Linné, -, *Systema naturae per regna tria naturae : secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Vol. 1, pt. 7 (13th edition)*. (Lugduni : Apud J. B. Delamolliere, 1789).
9. C. M. Lefèvre, J. A. Sharp, K. R. Nicholas, Evolution of Lactation: Ancient Origin and Extreme Adaptations of the Lactation System. *Annual Review of Genomics and Human Genetics* **11**, 219-238 (2010).
10. O. T. Oftedal, The mammary gland and its origin during synapsid evolution. *J Mammary Gland Biol Neoplasia* **7**, 225-252 (2002).
11. H. Macias, L. Hinck, Mammary gland development. *Wiley Interdiscip Rev Dev Biol* **1**, 533-557 (2012).
12. C. W. Daniel, G. H. Smith, The mammary gland: a model for development. *J Mammary Gland Biol Neoplasia* **4**, 3-8 (1999).
13. D. Ventrella *et al.*, Animal Models for In Vivo Lactation Studies: Anatomy, Physiology and Milk Compositions in the Most Used Non-Clinical Species: A Contribution from the ConcePTION Project. *Animals (Basel)* **11**, 714 (2021).
14. J. R. Hens, J. J. Wysolmerski, Key stages of mammary gland development: molecular mechanisms involved in the formation of the embryonic mammary gland. *Breast Cancer Res* **7**, 220-224 (2005).
15. J. M. Veltmaat, A. F. Ramsdell, E. Sterneck, Positional variations in mammary gland development and cancer. *J Mammary Gland Biol Neoplasia* **18**, 179-188 (2013).
16. G. W. Robinson, Cooperation of signalling pathways in embryonic mammary gland development. *Nat Rev Genet* **8**, 963-972 (2007).
17. C. J. Watson, W. T. Khaled, Mammary development in the embryo and adult: new insights into the journey of morphogenesis and commitment. *Development* **147**, (2020).
18. H. Parmar, G. R. Cunha, Epithelial-stromal interactions in the mouse and human mammary gland in vivo. *Endocr Relat Cancer* **11**, 437-458 (2004).
19. B. A. Howard, B. A. Gusterson, Human breast development. *J Mammary Gland Biol Neoplasia* **5**, 119-137 (2000).
20. K. Kratochwil, In vitro analysis of the hormonal basis for the sexual dimorphism in the embryonic development of the mouse mammary gland. *J Embryol Exp Morphol* **25**, 141-153 (1971).
21. R. Lyons, C. H. Li, R. E. Johnson, The hormonal control of mammary growth and lactation. *Recent Prog Horm Res* **14**, 219-248; discussion 248-254 (1958).
22. W. V. Ingman, S. A. Robertson, Mammary gland development in transforming growth factor beta1 null mutant mice: systemic and epithelial effects. *Biol Reprod* **79**, 711-717 (2008).
23. I. H. Russo, J. Russo, Pregnancy-induced changes in breast cancer risk. *J Mammary Gland Biol Neoplasia* **16**, 221-233 (2011).
24. C. Brisken, S. Duss, Stem cells and the stem cell niche in the breast: an integrated hormonal and developmental perspective. *Stem Cell Rev* **3**, 147-156 (2007).
25. M. D. Sternlicht, H. Kouros-Mehr, P. Lu, Z. Werb, Hormonal and local control of mammary branching morphogenesis. *Differentiation* **74**, 365-381 (2006).
26. J. E. Fata, V. Chaudhary, R. Khokha, Cellular turnover in the mammary gland is

correlated with systemic levels of progesterone and not 17beta-estradiol during the estrous cycle. *Biol Reprod* **65**, 680-688 (2001).

27. J. E. Visvader, Keeping abreast of the mammary epithelial hierarchy and breast tumorigenesis. *Genes Dev* **23**, 2563-2577 (2009).

28. G. B. Silberstein, Postnatal mammary gland morphogenesis. *Microsc Res Tech* **52**, 155-162 (2001).

29. Y. Cui *et al.*, Inactivation of Stat5 in mouse mammary epithelium during pregnancy reveals distinct functions in cell proliferation, survival, and differentiation. *Mol Cell Biol* **24**, 8037-8047 (2004).

30. R. S. Talhouk, J. R. Chin, E. N. Unemori, Z. Werb, M. J. Bissell, Proteinases of the mammary gland: developmental regulation in vivo and vectorial secretion in culture. *Development* **112**, 439-449 (1991).

31. G. W. Robinson, A. B. Karpf, K. Kratochwil, Regulation of mammary gland development by tissue interaction. *J Mammary Gland Biol Neoplasia* **4**, 9-19 (1999).

32. C. van Genderen *et al.*, Development of several organs that require inductive epithelial-mesenchymal interactions is impaired in LEF-1-deficient mice. *Genes Dev* **8**, 2691-2703 (1994).

33. I. Satokata *et al.*, Msx2 deficiency in mice causes pleiotropic defects in bone growth and ectodermal organ formation. *Nat Genet* **24**, 391-395 (2000).

34. J. Foley *et al.*, Parathyroid hormone-related protein maintains mammary epithelial fate and triggers nipple skin differentiation during embryonic breast development. *Development* **128**, 513-525 (2001).

35. G. R. Cunha *et al.*, Mammary phenotypic expression induced in epidermal cells by embryonic mammary mesenchyme. *Acta Anat (Basel)* **152**, 195-204 (1995).

36. B. S. Wiseman *et al.*, Site-specific inductive and inhibitory activities of MMP-2 and MMP-3 orchestrate mammary gland branching morphogenesis. *J Cell Biol* **162**, 1123-1133 (2003).

37. L. Szabova, S. S. Yamada, H. Birkedal-Hansen, K. Holmbeck, Expression pattern of four membrane-type matrix metalloproteinases in the normal and diseased mouse mammary gland. *J Cell Physiol* **205**, 123-132 (2005).

38. C. J. Ormandy *et al.*, Null mutation of the prolactin receptor gene produces multiple reproductive defects in the mouse. *Genes Dev* **11**, 167-178 (1997).

39. O. M. Conneely, B. Mulac-Jericevic, J. P. Lydon, F. J. De Mayo, Reproductive functions of the progesterone receptor isoforms: lessons from knock-out mice. *Mol Cell Endocrinol* **179**, 97-103 (2001).

40. N. D. Horseman *et al.*, Defective mammopoiesis, but normal hematopoiesis, in mice with a targeted disruption of the prolactin gene. *EMBO J* **16**, 6926-6935 (1997).

41. C. Brisken *et al.*, A paracrine role for the epithelial progesterone receptor in mammary gland development. *Proc Natl Acad Sci U S A* **95**, 5076-5081 (1998).

42. X. Liu *et al.*, Stat5a is mandatory for adult mammary gland development and lactogenesis. *Genes Dev* **11**, 179-186 (1997).

43. S. R. Oakes, R. L. Rogers, M. J. Naylor, C. J. Ormandy, Prolactin regulation of mammary gland development. *J Mammary Gland Biol Neoplasia* **13**, 13-28 (2008).

44. C. M. D'Cruz *et al.*, Persistent parity-induced changes in growth factors, TGF-beta3, and differentiation in the rodent mammary gland. *Mol Endocrinol* **16**, 2034-2051 (2002).

45. M. Li *et al.*, Mammary-derived signals activate programmed cell death during the first stage of mammary gland involution. *Proc Natl Acad Sci U S A* **94**, 3425-3430 (1997).

46. B. A. Creamer *et al.*, Stat5 promotes survival of mammary epithelial cells through transcriptional activation of a distinct promoter in Akt1. *Mol Cell Biol* **30**, 2957-2970 (2010).

47. K. L. Schwertfeger, M. M. Richert, S. M. Anderson, Mammary gland involution is delayed by activated Akt in transgenic mice. *Mol Endocrinol* **15**, 867-881 (2001).

48. J. N. Lilla, R. V. Joshi, C. S. Craik, Z. Werb, Active plasma kallikrein localizes to mast cells and regulates epithelial cell apoptosis, adipocyte differentiation, and stromal remodeling during mammary gland involution. *J Biol Chem* **284**, 13792-13803 (2009).

49. C. J. Sympson *et al.*, Targeted expression of stromelysin-1 in mammary gland provides evidence for a role of proteinases in

branching morphogenesis and the requirement for an intact basement membrane for tissue-specific gene expression. *J Cell Biol* **125**, 681-693 (1994).

50. N. Y. Fu, E. Nolan, G. J. Lindeman, J. E. Visvader, Stem Cells and the Differentiation Hierarchy in Mammary Gland Development. *Physiol Rev* **100**, 489-523 (2020).

51. L. Hennighausen, G. W. Robinson, Information networks in the mammary gland. *Nat Rev Mol Cell Biol* **6**, 715-725 (2005).

52. M. Shackleton *et al.*, Generation of a functional mammary gland from a single stem cell. *Nature* **439**, 84-88 (2006).

53. J. Stingl *et al.*, Purification and unique properties of mammary epithelial stem cells. *Nature* **439**, 993-997 (2006).

54. J. E. Visvader, J. Stingl, Mammary stem cells and the differentiation hierarchy: current status and perspectives. *Genes Dev* **28**, 1143-1158 (2014).

55. J. M. Williams, C. W. Daniel, Mammary ductal elongation: differentiation of myoepithelium and basal lamina during branching morphogenesis. *Dev Biol* **97**, 274-290 (1983).

56. R. C. Hovey, L. Aimo, Diverse and active roles for adipocytes during mammary gland growth and function. *J Mammary Gland Biol Neoplasia* **15**, 279-290 (2010).

57. J. C. Bartley, J. T. Emerman, M. J. Bissell, Metabolic cooperativity between epithelial cells and adipocytes of mice. *Am J Physiol* **241**, C204-208 (1981).

58. M. F. Gregor *et al.*, The role of adipocyte XBP1 in metabolic regulation during lactation. *Cell Rep* **3**, 1430-1439 (2013).

59. B. A. Howard, P. Lu, Stromal regulation of embryonic and postnatal mammary epithelial development and differentiation. *Semin Cell Dev Biol* **25-26**, 43-51 (2014).

60. M. Simian *et al.*, The interplay of matrix metalloproteinases, morphogens and growth factors is necessary for branching of mammary epithelial cells. *Development* **128**, 3117-3131 (2001).

61. B. S. Wiseman, Z. Werb, Stromal effects on mammary gland development and breast cancer. *Science* **296**, 1046-1049 (2002).

62. K. L. Betterman *et al.*, Remodeling of the lymphatic vasculature during mouse mammary gland morphogenesis is mediated via epithelial-derived lymphangiogenic stimuli. *Am J Pathol* **181**, 2225-2238 (2012).

63. N. Gjorevski, C. M. Nelson, Integrated morphodynamic signalling of the mammary gland. *Nat Rev Mol Cell Biol* **12**, 581-593 (2011).

64. V. Gouon-Evans, M. E. Rothenberg, J. W. Pollard, Postnatal mammary gland development requires macrophages and eosinophils. *Development* **127**, 2269-2282 (2000).

65. J. O'Brien, H. Martinson, C. Durand-Rougely, P. Schedin, Macrophages are crucial for epithelial cell death and adipocyte repopulation during mammary gland involution. *Development* **139**, 269-275 (2012).

66. J. M. Slack, Developmental biology of the pancreas. *Development* **121**, 1569-1580 (1995).

67. F. C. Pan, C. Wright, Pancreas organogenesis: from bud to plexus to gland. *Dev Dyn* **240**, 530-565 (2011).

68. H. P. Shih, A. Wang, M. Sander, Pancreas organogenesis: from lineage determination to morphogenesis. *Annu Rev Cell Dev Biol* **29**, 81-105 (2013).

69. L. Marty-Santos, O. Cleaver, Progenitor Epithelium: Sorting Out Pancreatic Lineages. *J Histochem Cytochem* **63**, 559-574 (2015).

70. A. Krapp *et al.*, The bHLH protein PTF1-p48 is essential for the formation of the exocrine and the correct spatial organization of the endocrine pancreas. *Genes Dev* **12**, 3752-3763 (1998).

71. M. H. Cleveland, J. M. Sawyer, S. Afelik, J. Jensen, S. D. Leach, Exocrine ontogenies: on the development of pancreatic acinar, ductal and centroacinar cells. *Semin Cell Dev Biol* **23**, 711-719 (2012).

72. T. Masui *et al.*, Replacement of Rbpj with Rbpjl in the PTF1 complex controls the final maturation of pancreatic acinar cells. *Gastroenterology* **139**, 270-280 (2010).

73. R. L. Beer, M. J. Parsons, M. Rovira, Centroacinar cells: At the center of pancreas regeneration. *Dev Biol* **413**, 8-15 (2016).

74. J. L. Kopp *et al.*, Identification of Sox9-dependent acinar-to-ductal reprogramming as the principal mechanism for initiation of

pancreatic ductal adenocarcinoma. *Cancer Cell* **22**, 737-750 (2012).

75. A. E. Schaffer, K. K. Freude, S. B. Nelson, M. Sander, Nkx6 transcription factors and Ptf1a function as antagonistic lineage determinants in multipotent pancreatic progenitors. *Dev Cell* **18**, 1022-1029 (2010).

76. M. Solar *et al.*, Pancreatic exocrine duct cells give rise to insulin-producing beta cells during embryogenesis but not after birth. *Dev Cell* **17**, 849-860 (2009).

77. D. Kopinke, L. C. Murtaugh, Exocrine-to-endocrine differentiation is detectable only prior to birth in the uninjured mouse pancreas. *BMC Dev Biol* **10**, 38 (2010).

78. C. D. Logsdon, B. Ji, The role of protein synthesis and digestive enzymes in acinar cell injury. *Nat Rev Gastroenterol Hepatol* **10**, 362-370 (2013).

79. N. M. Le Douarin, On the origin of pancreatic endocrine cells. *Cell* **53**, 169-171 (1988).

80. R. Arrojo e Drigo *et al.*, New insights into the architecture of the islet of Langerhans: a focused cross-species assessment. *Diabetologia* **58**, 2218-2228 (2015).

81. U. Dahl, A. Sjodin, H. Semb, Cadherins regulate aggregation of pancreatic beta-cells in vivo. *Development* **122**, 2895-2902 (1996).

82. J. C. Stendahl, D. B. Kaufman, S. I. Stupp, Extracellular matrix in pancreatic islets: relevance to scaffold design and transplantation. *Cell Transplant* **18**, 1-12 (2009).

83. N. K. Wessells, J. H. Cohen, Early Pancreas Organogenesis: Morphogenesis, Tissue Interactions, and Mass Effects. *Dev Biol* **15**, 237-270 (1967).

84. J. S. Burlison, Q. Long, Y. Fujitani, C. V. Wright, M. A. Magnuson, Pdx-1 and Ptf1a concurrently determine fate specification of pancreatic multipotent progenitor cells. *Dev Biol* **316**, 74-86 (2008).

85. Y. Guz *et al.*, Expression of murine STF-1, a putative insulin gene transcription factor, in beta cells of pancreas, duodenal epithelium and pancreatic exocrine and endocrine progenitors during ontogeny. *Development* **121**, 11-18 (1995).

86. Y. Kawaguchi *et al.*, The role of the transcriptional regulator Ptf1a in converting intestinal to pancreatic progenitors. *Nat Genet* **32**, 128-134 (2002).

87. D. Kopinke *et al.*, Lineage tracing reveals the dynamic contribution of Hes1+ cells to the developing and adult pancreas. *Development* **138**, 431-441 (2011).

88. B. Z. Stanger, A. J. Tanaka, D. A. Melton, Organ size is limited by the number of embryonic progenitor cells in the pancreas but not the liver. *Nature* **445**, 886-891 (2007).

89. S. K. Kim *et al.*, Activin receptor patterning of foregut organogenesis. *Genes Dev* **14**, 1866-1871 (2000).

90. M. Kumar, D. Melton, Pancreas specification: a budding question. *Curr Opin Genet Dev* **13**, 401-407 (2003).

91. M. Martin, V. Hauer, M. Messmer, C. Orvain, G. Gradwohl, Transcription factors in pancreatic development. Animal models. *Endocr Dev* **12**, 24-32 (2007).

92. L. C. Murtaugh, B. Z. Stanger, K. M. Kwan, D. A. Melton, Notch signaling controls multiple steps of pancreatic differentiation. *Proc Natl Acad Sci U S A* **100**, 14920-14925 (2003).

93. J. Hald *et al.*, Activated Notch1 prevents differentiation of pancreatic acinar cells and attenuate endocrine development. *Dev Biol* **260**, 426-437 (2003).

94. L. Zhu, G. Shi, C. M. Schmidt, R. H. Hruban, S. F. Konieczny, Acinar cells contribute to the molecular heterogeneity of pancreatic intraepithelial neoplasia. *Am J Pathol* **171**, 263-273 (2007).

95. S. Afelik *et al.*, Notch-mediated patterning and cell fate allocation of pancreatic progenitor cells. *Development* **139**, 1744-1753 (2012).

96. F. Esni *et al.*, Notch inhibits Ptf1 function and acinar cell differentiation in developing mouse and zebrafish pancreas. *Development* **131**, 4213-4224 (2004).

97. J. Magenheim *et al.*, Ngn3(+) endocrine progenitor cells control the fate and morphogenesis of pancreatic ductal epithelium. *Dev Biol* **359**, 26-36 (2011).

98. P. Jacquemin, F. P. Lemaigre, G. G. Rousseau, The Onecut transcription factor HNF-6 (OC-1) is required for timely specification of the pancreas and acts upstream of Pdx-1 in the specification cascade. *Dev Biol* **258**, 105-116 (2003).

99. R. Klinck *et al.*, A BAC transgenic Hes1-EGFP reporter reveals novel expression domains in mouse embryos. *Gene Expr Patterns* **11**, 415-426 (2011).

100. V. Vanhorenbeeck *et al.*, Role of the Onecut transcription factors in pancreas morphogenesis and in pancreatic and enteric endocrine differentiation. *Dev Biol* **305**, 685-694 (2007).

101. J. Wang *et al.*, Prox1 activity controls pancreas morphogenesis and participates in the production of "secondary transition" pancreatic endocrine cells. *Dev Biol* **286**, 182-194 (2005).

102. Q. Zhou *et al.*, A multipotent progenitor domain guides pancreatic organogenesis. *Dev Cell* **13**, 103-114 (2007).

103. S. R. Holmstrom *et al.*, LRH-1 and PTF1-L coregulate an exocrine pancreas-specific transcriptional network for digestive function. *Genes Dev* **25**, 1674-1679 (2011).

104. T. Masui, Q. Long, T. M. Beres, M. A. Magnuson, R. J. MacDonald, Early pancreatic development requires the vertebrate Suppressor of Hairless (RBPJ) in the PTF1 bHLH complex. *Genes Dev* **21**, 2629-2643 (2007).

105. M. Delous *et al.*, Sox9b is a key regulator of pancreaticobiliary ductal system development. *PLoS Genet* **8**, e1002754 (2012).

106. H. S. Kang *et al.*, Transcription factor Glis3, a novel critical player in the regulation of pancreatic beta-cell development and insulin gene expression. *Mol Cell Biol* **29**, 6366-6379 (2009).

107. M. A. Maestro *et al.*, Hnf6 and Tcf2 (MODY5) are linked in a gene network operating in a precursor cell domain of the embryonic pancreas. *Hum Mol Genet* **12**, 3307-3314 (2003).

108. C. E. Pierreux *et al.*, The transcription factor hepatocyte nuclear factor-6 controls the development of pancreatic ducts in the mouse. *Gastroenterology* **130**, 532-541 (2006).

109. H. P. Shih *et al.*, A Notch-dependent molecular circuitry initiates pancreatic endocrine and ductal cell differentiation. *Development* **139**, 2488-2499 (2012).

110. J. J. Westmoreland *et al.*, Pancreas-specific deletion of Prox1 affects development and disrupts homeostasis of the exocrine pancreas. *Gastroenterology* **142**, 999-1009.e1006 (2012).

111. H. Zhang *et al.*, Multiple, temporal-specific roles for HNF6 in pancreatic endocrine and ductal differentiation. *Mech Dev* **126**, 958-973 (2009).

112. T. M. Beres *et al.*, PTF1 is an organ-specific and Notch-independent basic helix-loop-helix complex containing the mammalian Suppressor of Hairless (RBP-J) or its paralogue, RBP-L. *Mol Cell Biol* **26**, 117-130 (2006).

113. B. Duvillié *et al.*, The mesenchyme controls the timing of pancreatic beta-cell differentiation. *Diabetes* **55**, 582-589 (2006).

114. G. K. Gittes, P. E. Galante, D. Hanahan, W. J. Rutter, H. T. Debase, Lineage-specific morphogenesis in the developing pancreas: role of mesenchymal factors. *Development* **122**, 439-447 (1996).

115. E. Bader *et al.*, Identification of proliferative and mature β-cells in the islets of Langerhans. *Nature* **535**, 430-434 (2016).

116. P. A. Seymour, M. Sander, Historical Perspective: Beginnings of the β-Cell. *Diabetes* **60**, 364 (2011).

117. S. I. Hajdu, A note from history: landmarks in history of cancer, part 2. *Cancer* **117**, 2811-2820 (2011).

118. R. A. Weinberg, *The biology of cancer*. (2016).

119. J. Gabriel, *The biology of cancer*. (John Wiley & Sons, Chichester ;, ed. 2nd ed., 2007).

120. D. Hanahan, R. A. Weinberg, The hallmarks of cancer. *Cell (Cambridge)* **100**, 57 (2000).

121. D. Hanahan, Robert a. Weinberg, Hallmarks of Cancer: The Next Generation. *Cell* **144**, 646-674 (2011).

122. D. Hanahan, R. A. Weinberg, Hallmarks of cancer: the next generation. *Cell* **144**, 646-674 (2011).

123. J. Campisi, Aging, cellular senescence, and cancer. *Annu Rev Physiol* **75**, 685-705 (2013).

124. F. Rodier, J. Campisi, Four faces of cellular senescence. *J Cell Biol* **192**, 547-556 (2011).

125. M. Milanovic *et al.*, Senescence-associated reprogramming promotes cancer stemness. *Nature* **553**, 96-100 (2018).

126. N. E. Sharpless, C. J. Sherr, Forging a signature of in vivo senescence. *Nat Rev Cancer* **15**, 397-408 (2015).

127. G. P. Dimri *et al.*, A biomarker that identifies senescent human cells in culture and in aging skin in vivo. *Proc Natl Acad Sci U S A* **92**, 9363-9367 (1995).

128. D. J. Kurz, S. Decary, Y. Hong, J. D. Erusalimsky, Senescence-associated (beta)-galactosidase reflects an increase in lysosomal mass during replicative ageing of human endothelial cells. *J Cell Sci* **113 (Pt 20)**, 3613-3622 (2000).

129. J. C. Acosta *et al.*, Chemokine signaling via the CXCR2 receptor reinforces senescence. *Cell* **133**, 1006-1018 (2008).

130. T. Kuilman *et al.*, Oncogene-induced senescence relayed by an interleukin-dependent inflammatory network. *Cell* **133**, 1019-1031 (2008).

131. J. P. Coppé *et al.*, Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. *PLoS Biol* **6**, 2853-2868 (2008).

132. F. Bray *et al.*, Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* **68**, 394-424 (2018).

133. M. Hidalgo *et al.*, Addressing the challenges of pancreatic cancer: future directions for improving outcomes. *Pancreatology* **15**, 8-18 (2015).

134. R. L. Siegel, K. D. Miller, A. Jemal, Cancer Statistics, 2017. *CA Cancer J Clin* **67**, 7-30 (2017).

135. A. J. Munoz Martin, J. Adeva, J. Martinez-Galan, J. J. Reina, M. Hidalgo, Pancreatic ductal adenocarcinoma: metastatic disease. *Clin Transl Oncol* **19**, 1423-1429 (2017).

136. D. S. Klimstra, Nonductal neoplasms of the pancreas. *Mod Pathol* **20 Suppl 1**, S94-112 (2007).

137. T. Muniraj, P. A. Jamidar, H. R. Aslanian, Pancreatic cancer: a comprehensive review and update. *Dis Mon* **59**, 368-402 (2013).

138. D. P. Ryan, T. S. Hong, N. Bardeesy, Pancreatic adenocarcinoma. *N Engl J Med* **371**, 2140-2141 (2014).

139. G. M. Petersen, Familial pancreatic cancer. *Semin Oncol* **43**, 548-553 (2016).

140. A. P. Klein *et al.*, Prospective risk of pancreatic cancer in familial pancreatic cancer kindreds. *Cancer Res* **64**, 2634-2638 (2004).

141. K. A. Brune *et al.*, Importance of age of onset in pancreatic cancer kindreds. *J Natl Cancer Inst* **102**, 119-126 (2010).

142. R. Z. Stolzenberg-Solomon, C. Schairer, S. Moore, A. Hollenbeck, D. T. Silverman, Lifetime adiposity and risk of pancreatic cancer in the NIH-AARP Diet and Health Study cohort. *Am J Clin Nutr* **98**, 1057-1065 (2013).

143. S. Solomon, S. Das, R. Brand, D. C. Whitcomb, Inherited pancreatic cancer syndromes. *Cancer J* **18**, 485-491 (2012).

144. J. Benzel, V. Fendrich, Familial Pancreatic Cancer. *Oncol Res Treat* **41**, 611-618 (2018).

145. M. S. Brose *et al.*, Cancer risk estimates for BRCA1 mutation carriers identified in a risk evaluation program. *J Natl Cancer Inst* **94**, 1365-1372 (2002).

146. H. F. Vasen *et al.*, Risk of developing pancreatic cancer in families with familial atypical multiple mole melanoma associated with a specific 19 deletion of p16 (p16-Leiden). *Int J Cancer* **87**, 809-811 (2000).

147. F. M. Walter *et al.*, Symptoms and patient factors associated with diagnostic intervals for pancreatic cancer (SYMPTOM pancreatic study): a prospective cohort study. *Lancet Gastroenterol Hepatol* **1**, 298-306 (2016).

148. M. Schmidt-Hansen, S. Berendse, W. Hamilton, Symptoms of Pancreatic Cancer in Primary Care: A Systematic Review. *Pancreas* **45**, 814-818 (2016).

149. U. K. Ballehaninna, R. S. Chamberlain, The clinical utility of serum CA 19-9 in the diagnosis, prognosis and management of pancreatic adenocarcinoma: An evidence based appraisal. *J Gastrointest Oncol* **3**, 105-119 (2012).

150. D. Li, K. Xie, R. Wolff, J. L. Abbruzzese, Pancreatic cancer. *Lancet* **363**, 1049-1057 (2004).

151. V. Corbo, G. Tortora, A. Scarpa, Molecular pathology of pancreatic cancer: from bench-to-bedside translation. *Curr Drug Targets* **13**, 744-752 (2012).

152. P. Ghaneh, E. Costello, J. P. Neoptolemos, Biology and management of pancreatic cancer. *Postgrad Med J* **84**, 478-497 (2008).

153. M. Porta *et al.*, Exocrine pancreatic cancer: symptoms at presentation and their relation

to tumour site and stage. *Clin Transl Oncol* **7**, 189-197 (2005).

154. E. A. Collisson *et al.*, Subtypes of pancreatic ductal adenocarcinoma and their differing responses to therapy. *Nat Med* **17**, 500-503 (2011).

155. R. A. Moffitt *et al.*, Virtual microdissection identifies distinct tumor- and stroma-specific subtypes of pancreatic ductal adenocarcinoma. *Nat Genet* **47**, 1168-1178 (2015).

156. A. c. society. (2017).

157. T. J. Grant, K. Hua, A. Singh, Molecular Pathogenesis of Pancreatic Cancer. *Prog Mol Biol Transl Sci* **144**, 241-275 (2016).

158. G. Bethune, D. Bethune, N. Ridgway, Z. Xu, Epidermal growth factor receptor (EGFR) in lung cancer: an overview and update. *J Thorac Dis* **2**, 48-51 (2010).

159. L. Cheng, A. Lopez-Beltran, F. Massari, G. T. MacLennan, R. Montironi, Molecular testing for BRAF mutations to inform melanoma treatment decisions: a move toward precision medicine. *Mod Pathol* **31**, 24-38 (2018).

160. N. Samuel, T. J. Hudson, The molecular and cellular heterogeneity of pancreatic ductal adenocarcinoma. *Nat Rev Gastroenterol Hepatol* **9**, 77-87 (2011).

161. B. Uzunparmak, I. H. Sahin, Pancreatic cancer microenvironment: a current dilemma. *Clin Transl Med* **8**, 2 (2019).

162. J. P. Neoptolemos *et al.*, Adjuvant chemoradiotherapy and chemotherapy in resectable pancreatic cancer: a randomised controlled trial. *Lancet* **358**, 1576-1585 (2001).

163. V. Vaccaro, I. Sperduti, M. Milella, FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. *N Engl J Med* **365**, 768-769; author reply 769 (2011).

164. A. Maitra, N. Fukushima, K. Takaori, R. H. Hruban, Precursors to invasive pancreatic cancer. *Adv Anat Pathol* **12**, 81-91 (2005).

165. N. Habbe *et al.*, Spontaneous induction of murine pancreatic intraepithelial neoplasia (mPanIN) by acinar cell targeting of oncogenic Kras in adult mice. *Proc Natl Acad Sci U S A* **105**, 18913-18918 (2008).

166. J. P. De La O *et al.*, Notch and Kras reprogram pancreatic acinar cells to ductal intraepithelial neoplasia. *Proc Natl Acad Sci U S A* **105**, 18907-18912 (2008).

167. C. Guerra *et al.*, Chronic pancreatitis is essential for induction of pancreatic ductal adenocarcinoma by K-Ras oncogenes in adult mice. *Cancer Cell* **11**, 291-302 (2007).

168. J. N. Jensen *et al.*, Recapitulation of elements of embryonic development in adult mouse pancreatic regeneration. *Gastroenterology* **128**, 728-741 (2005).

169. V. Fendrich *et al.*, Hedgehog signaling is required for effective regeneration of exocrine pancreas. *Gastroenterology* **135**, 621-631 (2008).

170. A. E. Arias, M. Bendayan, Differentiation of pancreatic acinar cells into duct-like cells in vitro. *Lab Invest* **69**, 518-530 (1993).

171. R. C. De Lisle, C. D. Logsdon, Pancreatic acinar cells in culture: expression of acinar and ductal antigens in a growth-related manner. *Eur J Cell Biol* **51**, 64-75 (1990).

172. P. A. Hall, N. R. Lemoine, Rapid acinar to ductal transdifferentiation in cultured human exocrine pancreas. *J Pathol* **166**, 97-103 (1992).

173. M. R. Vila, J. Lloreta, F. X. Real, Normal human pancreas cultures display functional ductal characteristics. *Lab Invest* **71**, 423-431 (1994).

174. S. Yuan, W. P. Duguid, D. Agapitos, B. Wyllie, L. Rosenberg, Phenotypic modulation of hamster acinar cells by culture in collagen matrix. *Exp Cell Res* **237**, 247-258 (1997).

175. J. L. Kopp *et al.*, Sox9+ ductal cells are multipotent progenitors throughout development but do not produce new endocrine cells in the normal or injured adult pancreas. *Development* **138**, 653-665 (2011).

176. G. Y. Liou *et al.*, Mutant KRas-Induced Mitochondrial Oxidative Stress in Acinar Cells Upregulates EGFR Signaling to Drive Formation of Pancreatic Precancerous Lesions. *Cell Rep* **14**, 2325-2336 (2016).

177. C. M. Ardito *et al.*, EGF receptor is required for KRAS-induced pancreatic tumorigenesis. *Cancer Cell* **22**, 304-317 (2012).

178. C. Navas *et al.*, EGF receptor signaling is essential for k-ras oncogene-driven pancreatic ductal adenocarcinoma. *Cancer Cell* **22**, 318-330 (2012).

179. C. D. Logsdon, B. Ji, Ras activity in acinar cells links chronic pancreatitis and

pancreatic cancer. *Clin Gastroenterol Hepatol* **7**, S40-43 (2009).

180. C. Guerra *et al.*, Pancreatitis-induced inflammation contributes to pancreatic cancer by inhibiting oncogene-induced senescence. *Cancer Cell* **19**, 728-739 (2011).

181. N. Bardeesy, R. A. DePinho, Pancreatic cancer biology and genetics. *Nat Rev Cancer* **2**, 897-909 (2002).

182. P. Storz, Acinar cell plasticity and development of pancreatic ductal adenocarcinoma. *Nat Rev Gastroenterol Hepatol* **14**, 296-304 (2017).

183. E. Manser, T. Leung, H. Salihuddin, Z. S. Zhao, L. Lim, A brain serine/threonine protein kinase activated by Cdc42 and Rac1. *Nature* **367**, 40-46 (1994).

184. S. Bagrodia, S. J. Taylor, C. L. Creasy, J. Chernoff, R. A. Cerione, Identification of a mouse p21Cdc42/Rac activated kinase. *J Biol Chem* **270**, 22731-22737 (1995).

185. R. Rathinam, A. Berrier, S. K. Alahari, Role of Rho GTPases and their regulators in cancer progression. *Front Biosci (Landmark Ed)* **16**, 2561-2571 (2011).

186. P. M. Chan, E. Manser, PAKs in human disease. *Prog Mol Biol Transl Sci* **106**, 171-187 (2012).

187. T. I. Strochlic, J. Viaud, U. E. Rennefahrt, T. Anastassiadis, J. R. Peterson, Phosphoinositides are essential coactivators for p21-activated kinase 1. *Mol Cell* **40**, 493-500 (2010).

188. G. Buchwald *et al.*, Conformational switch and role of phosphorylation in PAK activation. *Mol Cell Biol* **21**, 5179-5189 (2001).

189. M. Pirruccello *et al.*, A dimeric kinase assembly underlying autophosphorylation in the p21 activated kinases. *J Mol Biol* **361**, 312-326 (2006).

190. R. Kumar, R. Sanawar, X. Li, F. Li, Structure, biochemistry, and biology of PAK kinases. *Gene* **605**, 20-31 (2017).

191. Y. Baskaran, Y. W. Ng, W. Selamat, F. T. Ling, E. Manser, Group I and II mammalian PAKs have different modes of activation by Cdc42. *EMBO Rep* **13**, 653-659 (2012).

192. B. H. Ha *et al.*, Type II p21-activated kinases (PAKs) are regulated by an autoinhibitory pseudosubstrate. *Proc Natl Acad Sci U S A* **109**, 16107-16112 (2012).

193. R. Kumar, D. Q. Li, PAKs in Human Cancer Progression: From Inception to Cancer Therapeutic to Future Oncobiology. *Adv Cancer Res* **130**, 137-209 (2016).

194. M. Radu, G. Semenova, R. Kosoff, J. Chernoff, PAK signalling during the development and progression of cancer. *Nat Rev Cancer* **14**, 13-25 (2014).

195. K. M. Allen *et al.*, PAK3 mutation in nonsyndromic X-linked mental retardation. *Nat Genet* **20**, 25-30 (1998).

196. N. Gnesutta, A. Minden, Death receptor-induced activation of initiator caspase 8 is antagonized by serine/threonine kinase PAK4. *Mol Cell Biol* **23**, 7838-7848 (2003).

197. L. E. Arias-Romero, J. Chernoff, A tale of two Paks. *Biol Cell* **100**, 97-108 (2008).

198. J. Qu *et al.*, PAK4 kinase is essential for embryonic viability and for proper neuronal development. *Mol Cell Biol* **23**, 7122-7133 (2003).

199. M. G. Callow *et al.*, Requirement for PAK4 in the anchorage-independent growth of human cancer cell lines. *J Biol Chem* **277**, 550-558 (2002).

200. Z. Li *et al.*, p21-activated kinase 4 phosphorylation of integrin beta5 Ser-759 and Ser-762 regulates cell migration. *J Biol Chem* **285**, 23699-23710 (2010).

201. A. E. Dart, C. M. Wells, P21-activated kinase 4--not just one of the PAK. *Eur J Cell Biol* **92**, 129-138 (2013).

202. M. Zhao *et al.*, Pdx1-Cre-driven conditional gene depletion suggests PAK4 as dispensable for mouse pancreas development. *Sci Rep* **7**, 7031 (2017).

203. H. Zhang, Z. Li, E. K. Viklund, S. Strömblad, P21-activated kinase 4 interacts with integrin alpha v beta 5 and regulates alpha v beta 5-mediated cell migration. *J Cell Biol* **158**, 1287-1297 (2002).

204. Z. Li *et al.*, Integrin-mediated cell attachment induces a PAK4-dependent feedback loop regulating cell adhesion through modified integrin alpha v beta 5 clustering and turnover. *Mol Biol Cell* **21**, 3317-3329 (2010).

205. N. Gnesutta, J. Qu, A. Minden, The serine/threonine kinase PAK4 prevents caspase activation and protects cells from apoptosis. *J Biol Chem* **276**, 14414-14419 (2001).

206. X. Li, A. Minden, PAK4 functions in tumor necrosis factor (TNF) alpha-induced

survival pathways by facilitating TRADD binding to the TNF receptor. *J Biol Chem* **280**, 41192-41200 (2005).

207. Y. Tian, L. Lei, M. Cammarano, T. Nekrasova, A. Minden, Essential role for the Pak4 protein kinase in extraembryonic tissue development and vessel formation. *Mech Dev* **126**, 710-720 (2009).

208. Y. Tian, L. Lei, A. Minden, A key role for Pak4 in proliferation and differentiation of neural progenitor cells. *Dev Biol* **353**, 206-216 (2011).

209. T. Nekrasova, A. Minden, Role for p21-activated kinase PAK4 in development of the mammalian heart. *Transgenic Res* **21**, 797-811 (2012).

210. P. Rabieifar, T. Zhuang, T. D. F. Costa, M. Zhao, S. Strömblad, Normal mammary gland development after MMTV-Cre mediated conditional PAK4 gene depletion. *Sci Rep* **9**, 14436 (2019).

211. A. Begum *et al.*, Identification of PAK4 as a putative target gene for amplification within 19q13.12-q13.2 in oral squamous-cell carcinoma. *Cancer Sci* **100**, 1908-1916 (2009).

212. E. H. Mahlamäki *et al.*, High-resolution genomic and expression profiling reveals 105 putative amplification target genes in pancreatic cancer. *Neoplasia* **6**, 432-439 (2004).

213. S. J. Davis *et al.*, Functional analysis of genes in regions commonly amplified in high-grade serous and endometrioid ovarian cancer. *Clin Cancer Res* **19**, 1411-1421 (2013).

214. S. Chen *et al.*, Copy number alterations in pancreatic cancer identify recurrent PAK4 amplification. *Cancer Biol Ther* **7**, 1793-1802 (2008).

215. Y. Y. Jiang *et al.*, Targeting super-enhancer-associated oncogenes in oesophageal squamous cell carcinoma. *Gut* **66**, 1358-1368 (2017).

216. T. D. F. Costa *et al.*, PAK4 suppresses RELB to prevent senescence-like growth arrest in breast cancer. *Nat Commun* **10**, 3589 (2019).

217. M. K. Siu *et al.*, p21-activated kinase 4 regulates ovarian cancer cell proliferation, migration, and invasion and contributes to poor prognosis in patients. *Proc Natl Acad Sci U S A* **107**, 18622-18627 (2010).

218. T. Zhuang *et al.*, p21-activated kinase group II small compound inhibitor GNE-2861 perturbs estrogen receptor alpha signaling and restores tamoxifen-sensitivity in breast cancer cells. *Oncotarget* **6**, 43853-43868 (2015).

219. A. C. Kimmelman *et al.*, Genomic alterations link Rho family of GTPases to the highly invasive phenotype of pancreas cancer. *Proc Natl Acad Sci U S A* **105**, 19372-19377 (2008).

220. D. Li *et al.*, Activated Pak4 expression correlates with poor prognosis in human gastric cancer patients. *Tumour Biol* **36**, 9431-9436 (2015).

221. B. Song, W. Wang, Y. Zheng, J. Yang, Z. Xu, P21-activated kinase 1 and 4 were associated with colorectal cancer metastasis and infiltration. *J Surg Res* **196**, 130-135 (2015).

222. H. Tabusa, T. Brooks, A. J. Massey, Knockdown of PAK4 or PAK1 inhibits the proliferation of mutant KRAS colon cancer cells independently of RAF/MEK/ERK and PI3K/AKT signaling. *Mol Cancer Res* **11**, 109-121 (2013).

223. M. H. Park *et al.*, p21-Activated kinase 4 promotes prostate cancer progression through CREB. *Oncogene* **32**, 2475-2482 (2013).

224. D. Yeo, H. He, G. S. Baldwin, M. Nikfarjam, The role of p21-activated kinases in pancreatic cancer. *Pancreas* **44**, 363-369 (2015).

225. J. J. Park *et al.*, The p21-activated kinase 4-Slug transcription factor axis promotes epithelial-mesenchymal transition and worsens prognosis in prostate cancer. *Oncogene* **37**, 5147-5159 (2018).

226. Y. Li *et al.*, Nucleo-cytoplasmic shuttling of PAK4 modulates β-catenin intracellular translocation and signaling. *Biochim Biophys Acta* **1823**, 465-475 (2012).

227. C. Dan, A. Kelly, O. Bernard, A. Minden, Cytoskeletal changes regulated by the PAK4 serine/threonine kinase are mediated by LIM kinase 1 and cofilin. *J Biol Chem* **276**, 32115-32121 (2001).

228. V. DesMarais, M. Ghosh, R. Eddy, J. Condeelis, Cofilin takes the lead. *J Cell Sci* **118**, 19-26 (2005).

229. C. Almoguera *et al.*, Most human carcinomas of the exocrine pancreas contain mutant c-K-ras genes. *Cell* **53**, 549-554 (1988).

230. N. E. Mills, C. L. Fishman, W. N. Rom, N. Dubin, D. R. Jacobson, Increased

prevalence of K-ras oncogene mutations in lung adenocarcinoma. *Cancer Res* **55**, 1444-1447 (1995).

231. I. S. Boughdady, A. R. Kinsella, N. Y. Haboubi, P. F. Schofield, K-ras gene mutations in adenomas and carcinomas of the colon. *Surgical oncology.* **1**, 275-282 (1992).

232. R. L. Perlman, Mouse models of human disease: An evolutionary perspective. *Evol Med Public Health* **2016**, 170-176 (2016).

233. R. H. Waterston *et al.*, Initial sequencing and comparative analysis of the mouse genome. *Nature* **420**, 520-562 (2002).

234. L. L. Peters *et al.*, The mouse as a model for human biology: a resource guide for complex trait analysis.

235. K. Paigen, One hundred years of mouse genetics: an intellectual history. I. The classical period (1902-1980). *Genetics* **163**, 1-7 (2003).

236. M. R. Capecchi, Generating mice with targeted mutations. *Nat Med* **7**, 1086-1090 (2001).

237. O. Smithies, Forty years with homologous recombination. *Nat Med* **7**, 1083-1086 (2001).

238. C. Albanese, J. Hulit, T. Sakamaki, R. G. Pestell, Recent advances in inducible expression in transgenic mice. *Semin Cell Dev Biol* **13**, 129-141 (2002).

239. H. Kim, M. Kim, S. K. Im, S. Fang, Mouse Cre-LoxP system: general principles to determine tissue-specific roles of target genes. *Lab Anim Res* **34**, 147-159 (2018).

240. O. Yalcin-Ozuysal *et al.*, Antagonistic roles of Notch and p63 in controlling mammary epithelial cell fates. *Cell Death Differ* **17**, 1600-1612 (2010).

241. M. Gannon, P. L. Herrera, C. V. Wright, Mosaic Cre-mediated recombination in pancreas using the pdx-1 enhancer/promoter. *Genesis* **26**, 143-144 (2000).

242. K. U. Wagner *et al.*, Spatial and temporal expression of the Cre gene under the control of the MMTV-LTR in different lines of transgenic mice. *Transgenic Res* **10**, 545-553 (2001).

243. T. Vargo-Gogola, J. M. Rosen, Modelling breast cancer: one size does not fit all. *Nat Rev Cancer* **7**, 659-672 (2007).

244. P. Taneja *et al.*, MMTV mouse models and the diagnostic values of MMTV-like sequences in human breast cancer. *Expert Rev Mol Diagn* **9**, 423-440 (2009).

245. W. J. Muller, E. Sinn, P. K. Pattengale, R. Wallace, P. Leder, Single-step induction of mammary adenocarcinoma in transgenic mice bearing the activated c-neu oncogene. *Cell* **54**, 105-115 (1988).

246. D. Henrard, S. R. Ross, Endogenous mouse mammary tumor virus is expressed in several organs in addition to the lactating mammary gland. *J Virol* **62**, 3046-3049 (1988).

247. G. W. Robinson, L. Hennighausen, MMTV-Cre transgenes can adversely affect lactation: considerations for conditional gene deletion in mammary tissue. *Anal Biochem* **412**, 92-95 (2011).

248. B. A. Teicher, *Tumor Models in Cancer Research*. Cancer Drug Discovery and Development (Humana Press, Totowa, NJ, ed. 2nd ed. 2011., 2011).

249. M. Herreros-Villanueva, E. Hijona, A. Cosme, L. Bujanda, Mouse models of pancreatic cancer. *World J Gastroenterol* **18**, 1286-1294 (2012).

250. S. R. Hingorani *et al.*, Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse. *Cancer Cell* **4**, 437-450 (2003).

251. C. Guerra, M. Barbacid, Genetically engineered mouse models of pancreatic adenocarcinoma. *Mol Oncol* **7**, 232-247 (2013).

252. D. J. Cheon, S. Orsulic, Mouse models of cancer. *Annu Rev Pathol* **6**, 95-119 (2011).

253. N. Levy, The use of animal as models: ethical considerations. *Int J Stroke* **7**, 440-442 (2012).

254. F. Schwenk, U. Baron, K. Rajewsky, A cre-transgenic mouse strain for the ubiquitous deletion of loxP-flanked gene segments including deletion in germ cells. *Nucleic Acids Res* **23**, 5080-5081 (1995).

255. A. Abo *et al.*, PAK4, a novel effector for Cdc42Hs, is implicated in the reorganization of the actin cytoskeleton and in the formation of filopodia. *EMBO J* **17**, 6527-6540 (1998).

256. M. A. Furnari, M. L. Jobes, T. Nekrasova, A. Minden, G. C. Wagner, Functional deficits in PAK5, PAK6 and PAK5/PAK6 knockout mice. *PLoS One* **8**, e61321 (2013).

257. T. Nekrasova, M. L. Jobes, J. H. Ting, G. C. Wagner, A. Minden, Targeted disruption of the Pak5 and Pak6 genes in mice leads to deficits in learning and locomotion. *Dev Biol* **322**, 95-108 (2008).

258. Z. Wang, E. Oh, D. W. Clapp, J. Chernoff, D. C. Thurmond, Inhibition or ablation of p21-activated kinase (PAK1) disrupts glucose homeostatic mechanisms in vivo. *J Biol Chem* **286**, 41359-41367 (2011).

259. J. Piccand *et al.*, Pak3 promotes cell cycle exit and differentiation of β-cells in the embryonic pancreas and is necessary to maintain glucose homeostasis in adult mice. *Diabetes* **63**, 203-215 (2014).

260. J. W. Lee, C. A. Komar, F. Bengsch, K. Graham, G. L. Beatty, Genetically Engineered Mouse Models of Pancreatic Cancer: The KPC Model (LSL-Kras(G12D/+) ;LSL-Trp53(R172H/+) ;Pdx-1-Cre), Its Variants, and Their Application in Immuno-oncology Drug Discovery. *Curr Protoc Pharmacol* **73**, 14.39.11-14.39.20 (2016).

261. X. Deschênes-Simard *et al.*, Circumventing senescence is associated with stem cell properties and metformin sensitivity. *Aging Cell* **18**, e12889 (2019).

262. A. Kreso, J. E. Dick, Evolution of the cancer stem cell model. *Cell Stem Cell* **14**, 275-291 (2014).

263. C. H. Wong, Y. J. Li, Y. C. Chen, Therapeutic potential of targeting acinar cell reprogramming in pancreatic cancer. *World J Gastroenterol* **22**, 7046-7057 (2016).

264. A. F. Hezel, A. C. Kimmelman, B. Z. Stanger, N. Bardeesy, R. A. Depinho, Genetics and biology of pancreatic ductal adenocarcinoma. *Genes Dev* **20**, 1218-1249 (2006).

265. A. B. Lowenfels *et al.*, Pancreatitis and the risk of pancreatic cancer. International Pancreatitis Study Group. *N Engl J Med* **328**, 1433-1437 (1993).

266. D. C. Whitcomb, K. Pogue-Geile, Pancreatitis as a risk for pancreatic cancer. *Gastroenterol Clin North Am* **31**, 663-678 (2002).

267. B. M. Yoo *et al.*, Novel antioxidant ameliorates the fibrosis and inflammation of cerulein-induced chronic pancreatitis in a mouse model. *Pancreatology* **5**, 165-176 (2005).

268. C. E. Binkley *et al.*, The molecular basis of pancreatic fibrosis: common stromal gene expression in chronic pancreatitis and pancreatic adenocarcinoma. *Pancreas* **29**, 254-263 (2004).

269. M. V. Apte *et al.*, Desmoplastic reaction in pancreatic cancer: role of pancreatic stellate cells. *Pancreas* **29**, 179-187 (2004).

270. A. J. Rice *et al.*, Matrix stiffness induces epithelial-mesenchymal transition and promotes chemoresistance in pancreatic cancer cells. *Oncogenesis* **6**, e352 (2017).

271. B. W. Murray *et al.*, Small-molecule p21-activated kinase inhibitor PF-3758309 is a potent inhibitor of oncogenic signaling and tumor growth. *Proc Natl Acad Sci U S A* **107**, 9446-9451 (2010).

272. A. Aboukameel *et al.*, Novel p21-Activated Kinase 4 (PAK4) Allosteric Modulators Overcome Drug Resistance and Stemness in Pancreatic Ductal Adenocarcinoma. *Mol Cancer Ther* **16**, 76-87 (2017).