From the Department of Oncology-Pathology
Karolinska Institutet, Stockholm, Sweden

THE SECRET LIFE OF NUCLEAR IGF-1R: FUNCTIONS BEYOND TRADITIONAL SIGNALING PATHWAYS

DUDI WARSITO

Karolinska Institutet

Stockholm 2014

Published by Karolinska Institutet.
Printed by Åtta.45 Tryckeri AB

ISBN 978-91-7549-662-7

The secret life of nuclear IGF-1R: functions beyond traditional signaling pathways

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Dudi Warsito

Principal Supervisor:
Professor Olle Larsson
Karolinska Institutet
Department of Oncology-Pathology

Co-supervisor(s):
Dr. Bita Sehat
Karolinska Institutet
Department of Oncology-Pathology

Dr. Yingbo Lin
Karolinska Institutet
Department of Oncology-Pathology

Opponent:
Professor Rosemary O'Connor
University College Cork
School of Biochemistry and Cell Biology

Examination Board:
Docent Annika Wallberg
Karolinska Institutet
Department of Environmental Medicine

Professor Dan Grandér
Karolinska Institutet
Department of Oncology-Pathology

Professor Tore Bengtsson
Stockholm University
Department of Molecular Biosciences

ABSTRACT

The implications of IGF-1R in normal development and disease are well established. IGF-1R plays pivotal roles in cell proliferation and cell survival and aberrant activation result in tumorigenesis and cancer progression. Extensive studies have concluded that much of the biological effects of IGF-1R are mediated by a plethora of cytoplasmic signaling pathways originating from the cell surface. However, findings describing IGF-1R in the cell nucleus of cancer cells have recently begun to emerge. The high prevalence of IGF-1R in the nucleus of cancer cells suggests a functional role for nuclear IGF-1R in cancer biology.

The first study (paper I) in this thesis aimed to elucidate the role of nuclear IGF-1R in gene transcription. We show that nuclear IGF-1R associates with β-catenin and LEF-1, key components of the Wnt signaling pathway. Nuclear IGF-1R is enriched at the *cyclin D1* promoter and elevates cyclin D1 and axin2 protein levels.

In paper II we propose a model for IGF-1R nuclear transportation in cancer cells. We present data showing that the transportation is dependent on microtubules and the retrograde transport protein complex dynactin. IGF-1R was also found to associate with EEA1 in both the cytoplasm and the nucleus, suggesting that IGF-1R is membrane bound during the transport. By utilizing siRNA and mutant constructs we show that passage of IGF-1R across the nuclear envelope is dependent on importin-β, RanBP2 and Ran GTPase. The nuclear pore complex protein and SUMO E3 ligase, RanBP2, SUMOylates IGF-1R at the nuclear periphery and as a consequence stabilize IGF-1R. Stabilized receptor is able to enter the nucleus.

In the third paper we identified histone H3 as an interacting partner for nuclear IGF-1R. Furthermore, we show that IGF-1R phosphorylates histone H3Y41. By utilizing wild type and mutant histone H3 constructs we demonstrate that phosphorylated H3Y41 stabilizes the association of Brg1 chromatin remodeling protein to chromatin. We also identified *SNAI2* to be a target gene for nuclear IGF-1R and its expression was reduced when H3Y41 phosphorylation was impaired. Both IGF-1R and Brg1 was found to associate with *SNAI2* promoter.

Taken together, these studies provide novel findings about IGF-1R function and trafficking. We show that nuclear IGF-1R takes a more direct part in gene transcription in addition to its classical role as a cell surface receptor in cancer cells. We also propose a mechanism by which IGF-1R is transported into the nucleus. As IGF-1R is highly implicated in cell proliferation and cell survival, our findings provide a regulatory role for nuclear IGF-1R in tumorigenesis and cancer progression.

Our imagination is nothing compared with nature's awesome reality.

-Neil deGrasse Tyson

LIST OF SCIENTIFIC PAPERS

I. **Warsito D**, Sjöström S, Andersson S, Larsson O and Sehat B. Nuclear IGF1R is a transcriptional co-activator of LEF1/TCF. *EMBO Rep,* 13(3), 244-250, 2012.

II. Packham S, **Warsito D**, Lin Y, Sadi S, Karlsson R, Sehat B and Larsson O. Nuclear translocation of IGF-1R via $p150^{Glued}$ and an importin-β/RanBP2-dependent pathway in cancer cells. *Oncogene,* [Epub ahead of print], 2014.

III. Warsito D, Gnirck AC, Sehat B and Larsson O. Nuclear IGF-1R phosphorylates histone H3Y41 and induces *SNAI2* expression via Brg1 chromatin remodeling protein. *Manuscript.*

CONTENTS

LIST OF ABBREVIATIONS

AKT	Protein kinase B
ATP	Adenosine triphosphate
Brg1	Brahma related gene 1
c-Met	Hepatocyte growth factor receptor
ChIP	Chromatin immunoprecipitation
DNA	Deoxyribonucleic acid
E1	SUMO activating enzyme
E2	SUMO conjugating enzyme
E3	SUMO ligase
EEA1	Early endosomal antigen 1
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
eIF4E	Eukaryotic translational initiation factor 4E
ERAD	Endoplasmatic reticulum associated protein degradation
ERK	Extracellular signal regulated kinases
FGF	Fibroblast growth factor
FGFR	Fibroblast growth factor receptor
GAP	GTPase activating protein
GDP	Guanosine diphosphate
GSK3	Glycogen synthase kinase 3
GTP	Guanosine triphosphate
GEF	Guanine nucleotide exchange factor
IGF	Insulin-like growth factor
IGF-1R	Insulin-like growth factor 1 receptor
IGFBP	IGF binding proteins
IRS	Insulin receptor substrate
kDa	kilo Dalton
LEF	Lymphoid enhancer factor
Lys	Lysine
MAPK	Mitogen-activated protein kinase

mTOR	Mammalian target of rapamycin
mTORC1	Mammalian target of rapamycin complex 1
NEM	N-ethylmaleimide
NGFR	Nerve growth factor receptor
NLS	Nuclear localizing signal
NPC	Nuclear pore complex
PDGF	Platelet-derived growth factor
PDK	Phopshoinositide-dependent protein kinase
PI3K	Phosphatidyl-3'-kinase
PIP2	Phosphatidyl 3,4-diphoshpate
PIP3	Phosphatidyl 3,4,5-triphosphate
PP2A	Protein phosphatese 2A
PRAS40	Proline-rich Akt substrate 40 kDa
PTB	Phosphotyrosine binding domain
PTEN	Phosphatase and tensin homolog
RanBP2	Ran binding protein 2
RTK	Receptor tyrosine kinase
S6K1	Ribosomal protein S6 kinase beta-1
Ser	Serine
SH2	Src homology 2
Shc	Src homology and collagen
siRNA	Small interfering RNA
SUMO	Small-ubiquitin related modifier
TCF	T-cell factor
Thr	Threonine
Tyr	Tyrosine
TSM	Triple SUMO mutant
Wt	Wild type

1 INTRODUCTION

1.1 CANCER

Cancer is a highly complex disease that is characterized by uncontrolled cell growth and proliferation. Cancer was previously solely regarded as a genetic disease, but we now know that the development of cancer is dependent on the accumulation of genetic and epigenetic changes, which abolishes the regulatory machinery that governs normal cell growth and proliferation.

Though cancer is highly complex, in 2000 Hanahan and Weinberg proposed six traits that defined all cancers [1]:

1. Self-sufficiency of growth signals
2. Insensitivity to anti-growth signals
3. Evading apoptosis
4. Limitless replicative potential
5. Sustained angiogenesis
6. Tissue invasion and metastasis

A decade later this list was updated [2] with four additional traits:

7. Deregulated metabolism
8. Avoid immune destruction
9. Tumor-promoting inflammation
10. Genome instability

Many of the genes that are involved in these traits and thus promote tumorigenesis can be divided into three groups: proto-oncogenes, tumor suppressor genes and maintenance/caretaker genes [3, 4]. Proto-oncogenes, which are termed oncogenes when aberrantly expressed, promote cell proliferation and inhibit apoptosis. Tumor suppressor genes on the other hand have the opposite function by blocking cell proliferation and promote apoptosis. In cancer cells tumor suppressor genes are often inactivated, rendering the cells capable of evading apoptosis and sustaining proliferation. The third group of genes, caretaker genes, is part of the DNA repair machinery. The caretaker genes safeguard against sustained errors in the DNA that can arise during DNA replication. Errors that are not repaired result in genome instability, aneuploidy and aberrant expression or inactivation of oncogenes and tumor suppressor genes respectively [5-8].

Of the ten traits that were proposed by Hanahan and Weinberg, self-sufficiency of growth signals will be the main focus of this thesis. One group of proteins that has a major role in providing growth signals is the receptor tyrosine kinases. In the following sections I will discuss receptor tyrosine kinases' mode of action and regulation, with an emphasis on the insulin-like growth factor 1 receptor (IGF-1R). In the end I will present new data about nuclear IGF-1R function and trafficking.

1.2 RECEPTOR TYROSINE KINASES

Receptor tyrosine kinases (RTKs) are transmembrane cell surface receptors, which have fundamental roles in governing basic cellular processes such as proliferation, migration, differentiation and cell survival [9, 10]. They connect extracellular signals with nuclear events via a cascade of cytoplasmic signaling pathways.

Structurally RTKs constitute of an extracellular ligand-binding domain, a single-pass transmembrane domain, intracellular tyrosine kinase domain and a C-terminal domain. In humans there are 58 RTKs divided into 20 subfamilies (Figure 1). While the intracellular tyrosine kinase domain is similar among the RTKs [9, 11], the extracellular domain exhibit structural differences accounted by the various conserved elements that can be found in each receptor subfamily [9].

Activation and signaling through RTKs is initiated when a ligand (often a growth factor) binds the receptor in the extracellular ligand-binding domain. This induces receptor dimerization and trans-autophosphorylation where the tyrosine kinase domain phosphorylates its dimeric partner [12, 13]. The initial phosphorylation event results in a conformational change of the intracellular part where it adopts an active conformation allowing further phosphorylation [14, 15]. These conformational changes vary among the receptors and can occur at the juxtamembrane region, kinase region or C-terminal region [16-18]. The phosphorylated residues function as binding sites for Src homology 2 (SH2) and phospho tyrosine binding (PTB) domain containing adapter proteins [19], which relays the signal via cytoplasmic signaling pathways such as MAPK/Akt (mitogen activated protein kinase) and PI3K/Erk (phosphatidylinositol-3 kinase/extracellular signal-regulated kinases) pathways. Once the receptor has been activated, it undergoes endocytosis. The endocytosed receptor can then either be destined for lysosomal degradation to attenuate the signal or recycled back to the plasma membrane for another round of activation. This is a common mechanism for regulating RTK activity. However, as will be discussed in a later section, RTKs can be destined to other subcellular compartments.

The signal that originated from the extracellular region reaches the nucleus to either activate or inactivate genes involved in cellular processes such as cell proliferation, differentiation and migration. It is thus not surprising that aberrant expression and signaling through RTKs are major contributors to tumorigenesis [20-23]. The signaling pathways activated by RTKs, are however not as linear as what was previously thought. RTKs display extensive cross-talk with each other [24-26], other types of receptors [27-29] and signaling pathways [30, 31], which accounts for much of the outcomes that are initiated by the RTK. Furthermore, these extensive cross talks have been a major contributing factor for the development of drug resistance in cancer patients [32].

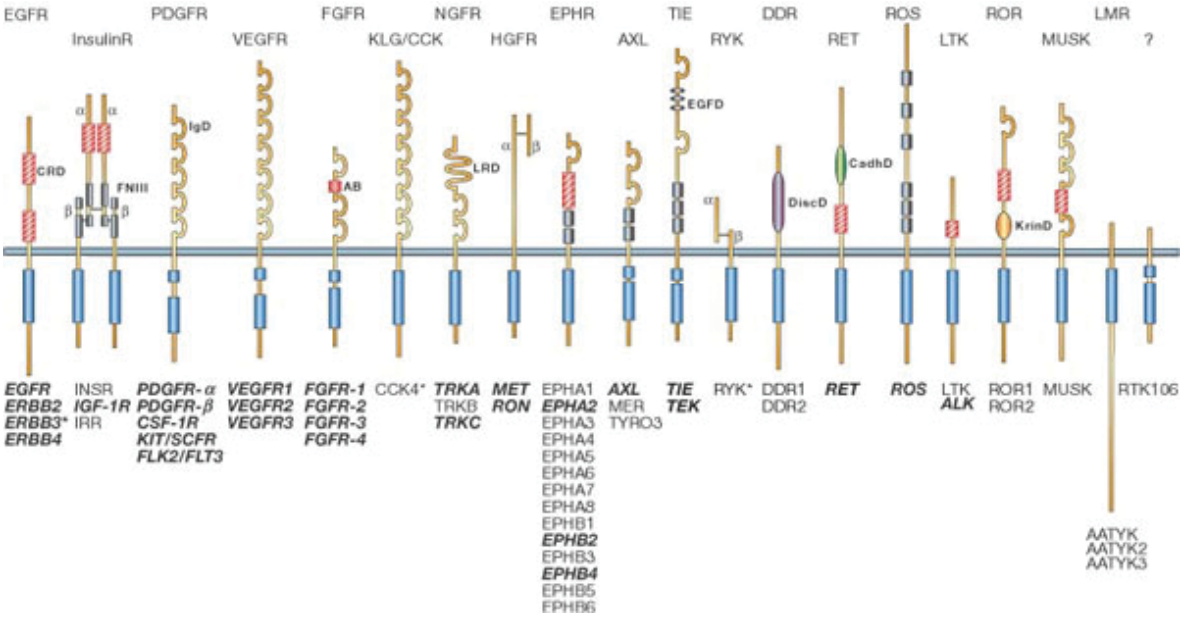

Figure 1: Human receptor tyrosine kinases. The prototypic receptor for each family is indicated above the receptor, and the known members are listed below. Abbreviations of the prototypic receptors: EGFR, epidermal growth factor receptor; InsR, insulin receptor; PDGFR, platelet-derived growth factor receptor; VEGFR; vascular endothelial growth factor receptor; FGFR, fibroblast growth factor receptor; KLG/CCK, colon carcinoma kinase; NGFR, nerve growth factor receptor; HGFR, hepatocyte growth factor receptor, EphR, ephrin receptor; Axl, a Tyro3 PTK; TIE, tyrosine kinase receptor in endothelial cells; RYK, receptor related to tyrosine kinases; DDR, discoidin domain receptor; Ret, rearranged during transfection; ROS, RTK expressed in some epithelial cell types; LTK, leukocyte tyrosine kinase; ROR, receptor orphan; MuSK, muscle-specific kinase; LMR, Lemur. Other abbreviations: AB, acidic box; CadhD, cadherin-like domain; CRD, cysteine-rich domain; DiscD, discoidin-like domain; EGFD, epidermal growth factor-like domain; FNIII, fibronectin type III-like domain; IgD, immunoglobulin-like domain; KrinD, kringle-like domain; LRD, leucine-rich domain. The symbols α and β denote distinct RTK subunits. RTK members in bold and italic type are implicated in human malignancies. An asterisk indicates that the member is devoid of intrinsic kinase activity. (Blume-Jensen and Hunter; *Nature* 2001, vol 411)

1.3 WNT SIGNALING PATHWAY

One major signaling pathway that has shown extensive cross talk with RTKs is the Wnt signaling pathway. The Wnt signaling pathway is an evolutionary conserved pathway that controls cell growth, differentiation and migration during embryonic development and self-renewal in adult tissues [33-36]. Aberrant Wnt activation is a major cause for several human pathologies including cancer [34].

The Wnt1 (Int-1 in mouse) ligand was discovered as an oncogene that could be activated by the integration of mouse mammary tumor virus in breast tumors [37]. Two years prior, Nüsslein-Volhard and Wieshaus, had discovered *Wingless* (Wg), a gene controlling segment polarity in larval development in *Drosophila* [38] that later on was shown to be a homolog of Int-1 [39]. Mammals have a total of 20 Wnt proteins grouped into 12 conserved subfamilies. Wnt proteins are about 40 kDa in size and are highly hydrophobic due to extensive lipid modifications by glycosylation and palmityolation [40, 41]. The lipid modifications of Wnt proteins are important for their signaling capabilities [42] and the binding to their receptors, Frizzled [43]. There are 10 Frizzled receptors in mammals. Frizzled are seven-pass transmembrane receptors with extracellular N-terminal cysteine-rich domains that provide most of the contacts with Wnt ligands [44]. In addition to Frizzled receptors, Wnt proteins also make contact with Lrp5/6 co-receptors. Lrp5/6 are single-pass transmembrane receptors and work together with Frizzled receptors in mediating Wnt signals [45, 46].

In absence of Wnt ligands, a cytoplasmic destruction complex targets the transcriptional co-activator β-catenin for destruction. The destruction complex constitutes of the tumor suppressor proteins Axin and APC (Adenomatous polyposis coli), and two constitutively active serine/threonine kinases GSK3 (Glycogen synthase kinase 3) and CK1 (Casein Kinase 1). The two kinases sequentially phosphorylate β-catenin, which is then recognized by the E3 ubiquitin ligase β-TrCP. As a consequence, β-catenin is ubiquitinated and targeted for proteosomal destruction, preventing nuclear accumulation of β-catenin and activation of target genes [47]. In presence of Wnt ligands, Axin is recruited to the plasma membrane, causing a dissociation of the destruction complex. This results in the release and stabilization of β-catenin. Stabilized β-catenin accumulates in the nucleus where it forms an active transcriptional complex with TCF/LEF-1 (T-cell factor/lymphoid enhancer factor-1) family of transcription factors by displacing the inhibitor Groucho [48, 49]. Active β-catenin/TCF/LEF-1 transcriptional complex activates a number of genes such as *cyclin D1* [50], *AXIN2* [51] and *c-MYC* [52].

In addition to its role as a transcriptional co-activator upon Wnt activation, β-catenin also has a cell adhesive function in epithelial cells. Here β-catenin is associated with the cytoplasmic tales of cadherins, most notably E-cadherin [53]. The association with E-cadherin is important to maintain epithelial integrity and disruption of this association by tyrosine phosphorylation of β-catenin is the initial steps of epithelial-mesenchymal transition [54].

As already mentioned several studies have provided evidence showing extensive interactions between RTKs and components of the Wnt signaling pathway. Playford *et al.* demonstrated that C10 colorectal cancer cell lines treated with IGF-1 resulted in the stabilization and nuclear accumulation of β-catenin [30]. In another study it was shown that insulin stimulated rat L6 myotubes cells inhibited GSK3 activity through phosphorylation by protein kinase B (PKB). More recently Krecji *et al.* showed that FGFR2/3, EGFR and TrkA receptor tyrosine kinases could induce MAPK mediated phosphorylation of Lrp6 resulting in activation of Wnt pathway. The study also showed that these RTKs phosphorylated β-catenin at tyrosine 142, a phosphorylation site known to stabilize β-catenin and promote its nuclear accumulation [55].

1.4 NUCLEO-CYTOPLASMIC TRAFFICKING

In eukaryotic cells a double membrane barrier called nuclear envelope surrounds the genetic material forming the cell nucleus. Passage of proteins across the nuclear envelope and into the nucleus is critical for gene regulation and DNA replication. Proteins can enter the nucleus either through passive diffusion (proteins less than 40 kDa) or by active transport mediated by transport receptors through the nuclear pore complex.

1.4.1 Nuclear pore complex

Passage into the nucleus occurs through cylindrical bidirectional channels embedded in the nuclear envelope called the nuclear pore complex (NPC). NPCs are large protein complexes (up to 120 MDa) constituted by 30 different proteins in multiple copies known as nucleoporins. Nucleoporins provide the shape and strength of the NPC but also binding sites for proteins, which are being transported through the NPC. The central channel is constituted by nucleoporins with unstructured filaments, known as phenylalanine-glycine (FG) repeats, that extends into the channel. These filamentous nucleoporins are also found on the cytoplasmic face of the NPC. The FG-repeats binds transport receptors (karyopherins) carrying cargoes that are to the transported through the NPC. One such filamentous nucleoporin is RanBP2 (Ran binding protein 2). RanBP2 is a 358 kDa protein comprised of multiple domains for protein-protein interactions. In addition to its interactions with transport receptors through the FG-repeats, RanBP2 also binds to microtubules and the dynein-dynactin motor protein complex, thus linking RanBP2 to the retrograde protein transport machinery. Furthermore, RanBP2 has a SUMO E3 ligase domain at its C-terminal, mediating SUMOylation of proteins to be transported into the nucleus.

1.4.2 Transport receptors

Transport receptors, known as karyopherins, bind cargoes that are to be transported through the NPC in an energy dependent process. There are over 20 karyopherins in eukaryotes. Karyopherins that mediates nuclear import are called importins while those mediating export are called exportins. Importins are divided into importin-β and importin-α. Importin-β can either bind its cargo directly or via importin-α. Recognition and binding to the nuclear localizing signal of cargoes by importin-α is mediated by the multiple arginine rich motifs (armadillo repeats). Importin-β does not harbor any armadillo repeats however it does harbor

HEAT (huntingtin elongation factor) repeats, which are similar to armadillo repeats. The HEAT repeats mediate binding to the FG-repeats that extend from the filamentous nucleoporins and link the importin-cargo complex to the NPC. Since active transport through the NPC is an energy dependent process, importin-β also binds to Ran GTPases to drive the transport (discussed below).

1.4.3 Nuclear localizing signal

Proteins that are transported into the nucleus through the NPC normally harbors a signal peptide termed nuclear localizing signal (NLS), which is recognized by the importin proteins. Early evidence that a signal peptide was required for nuclear import came from studies on the molecular chaperone nucleoplasmin [56]. Protelolytic hydrolysis of its 50 C-terminal amino acids inhibited its nuclear accumulation and this stretch of amino acids alone could accumulate in the nucleus of *Xenopus laevis* oocytes. Although this study demonstrated that nuclear import was a selective process, it was not until studies on the SV40 large T antigen that the first NLS sequence was identified [57]. Kalderon *et al.* identified a seven amino acid sequence rich in basic amino acids, PKKKRKV, was required for nuclear import of cytoplasmic proteins [57]. It was later revealed that the 50 C-terminal amino acids that was required for nucleoplasmin nuclear import to contain an 18 amino acid sequence rich in basic amino acids [58]. These original NLS rich in basic amino acids are classified as classical/conventional NLS. Other NLS that do not follow this consensus of having a stretch of basic amino acids have been discovered and are referred to as non-classical/non-conventional NLS. The repertoire of NLS is further expanded with the presence of NLS sequences that are divided into several stretches of amino acids referred to as bipartite or tripartite NLS.

1.4.4 Nuclear import mechanism

Transport of proteins larger than 40 kDa through the NPC is an energy dependent process. The minimum requirement for such transport to take place is an NLS, importin-β and Ran GTPase. As mentioned earlier, recognition and binding of cargo protein is mediated either directly by importin-β or indirectly through importin-α. The importin-α/β-cargo complex dock with the FG-repeats of the NPC through the HEAT repeats of importin-β. Transport into the nucleus is dependent on the Ras-like GTPase Ran. Just like other GTPases, Ran GTPase is found in two forms, either GDP bound form or GTP bound form. Cytoplasmic Ran GTPase associates with the importin-α/β-cargo complex and enters the nucleus in its Ran-GDP form. In the nucleus, a Ran guanine nucleotide exchange factor (RanGEF) exchanges GDP for GTP. This causes the imported cargo to dissociate and be released into the nucleus. Ran-GTP together with the importin proteins is then exported back into the cytosol where Ran GTPase activating protein (RanGAP) hydrolyze GTP to GDP rendering Ran ready for another cycle of import. The enrichment of RanGAP in the cytosol and RanGEF in the nucleus generates a gradient across the cytosol-nucleus of Ran-GDP (cytosol) and Ran-GTP (nucleus). This gradient ensures directionality of nucleo-cytoplasmic transport.

Although nuclear import is generally dependent on NLS and importin proteins, other mechanisms exists that are independent of NLS and/or importin proteins. β-catenin is a protein, which does not harbor a NLS but still undergoes nucleo-cytoplasmic trafficking. As β-catenin associates with TCF/LEF-1 it was originally thought that β-catenin would piggyback on TCF/LEF-1, which has a NLS [59, 60]. Later studies however provided evidence that β-catenin could be imported into the nucleus independently of TCF/LEF-1 and importin proteins by its direct association with the NPC through its armadillo repeats [61, 62].

1.5 CHROMATIN STRUCTURE AND REGULATION

If all the DNA molecules in a single human cell would be stretched out, it would be 2 meters long. In order for this amount of DNA to fit into a single cell or let alone a nucleus, the DNA needs to be tightly packed into a structure called chromatin. Chromatin is the combination of DNA and proteins. Packaging of DNA into chromatin is highly hierarchical and occurs at several levels where each level results in a higher order of chromatin compaction. The high level of compaction not only enables the DNA to fit into a nucleus, but is also a safeguard against DNA damage and unwanted transcriptional activation by preventing transcription factors to access the DNA.

1.5.1 Nucleosome – the fundamental unit of chromatin

The nucleosome is the most fundamental unit of chromatin consisting of 147 bp of DNA wrapped around a histone octamer in a superhelical manner. Nucleosomes are linked together with approximately 20 bp of linker DNA forming a 10 nm in diameter fiber of nucleosomal arrays resembling a "beads-on-a-string" structure under electron microscope [63-65]. Stability of the nucleosome is mediated by internal histone-histone interactions and electrostatic and hydrogen bonds between DNA-histones [66-68]. The 10 nm fibers can be further folded into 30 nm fibers and higher order chromatin structure increasing the chromatin compaction [69].

1.5.2 Histones and their modifications

Histones are highly conserved proteins essential for chromatin structure and function. They are characterized by the large globular histone fold domain made up by three alpha helices connected by short loops [70]. The N-terminal portion constitute of an unstructured tail that is highly enriched in various posttranslational modifications [71]. Four core histone proteins H2A, H2B, H3 and H4 in duplicate make up the histone octamer. DNA/octamer interaction is provided by 14 weak contacts, mostly at A:T regions, which together provide high stability. These interactions are mediated by the hydrogen bonds of the histone folds and the phosphodiester backbone of DNA [67].

If DNA is tightly packaged into chromatin, how does the transcriptional machinery access the DNA? Chromatin is a highly dynamic structure, which in loosely terms can exist in two states, closed (heterochromatin) or open (euchromatin) state [72]. In the closed state, DNA is

tightly compacted and inaccessible to transcription factors while the situation is opposite when the chromatin is in the open state (less compacted and more accessible). The components of chromatin are highly modifiable by reversible posttranslational modifications (PTMs). The DNA can be methylated while histones are modified by PTMs including methylation, acetylation and phosphorylation. The notion that histones could be modified came from studies in the early 60s [73]. These modifications affect DNA-histone interactions impacting nucleosome integrity and chromatin structure. As a consequence nuclear events such as transcription and DNA replication are impacted as well [71]. Though previously thought that histone modifications solely occurred on their exposed N-terminal tails, several studies show modifications on the large globular domain too [74-76].

Of all the histone modifications that have been discovered, acetylation, phosphorylation and methylation are the best characterized ones. Acetylation/deacetylation is mediated by a number of histone acetyl transferases and histone deacetyl transferases. Transfer of an acetyl group to lysine side chain of histones neutralizes the lysine's positive charge thereby weakening the DNA/histone interactions. Histone phosphorylation/dephosphorylation, mediated by kinases/phosphatases, occurs on serine, threonine and tyrosine residues. Just like histone acetylation, addition of a phosphate group adds a negative charge to the histone, affecting the DNA/histone interactions. Methylation occurs primarily on lysine and arginine residues. Histone methylation was previously thought to be irreversible, but has been disproved since the discovery of lysine-specific demethylase 1 [77]. In contrast to histone acetylation and phosphorylation, histone methylation does not alter the charge of histone. However, histone lysines can be modified by mono-, di- or tri-methylation [78].

The list of histone modifications is long and still expanding and these modifications do not occur in isolation. There is extensive cross talk between histone modifications within the same histone [79-81] and modifications on different histones [82]. The extensive combinations of possible histone modifications have led to the term histone code or epigenetic code [83]. The general idea behind the histone code is that combinations of histone modifications recruit chromatin remodeling complexes and other proteins that alters chromatin structure (discussed in next section). Chromatin remodeling is thus not only dependent on alterations of electrostatic charge upon histone modifications, but also by active remodeling by chromatin remodeling complexes.

1.5.3 Chromatin remodeling complexes

The switch from heterochromatin to euchromatin is dependent on a process referred to as chromatin remodeling. This energy dependent process disrupts the contacts between DNA and histone, resulting in nucleosomal free DNA. There are two major classes of enzymes that participate in chromatin remodeling: histone modifiers and chromatin remodeling complexes. Histone modifiers add posttranslational modifications to the histone proteins (discussed in previous section) and include kinases, acetyltransferases, methyltransferases and others.

Chromatin remodeling complexes are large protein complexes that disrupt the DNA/histone interactions. There are four families of chromatin remodeling complexes:

- SWI/SNF (switching defective/sucrose nonfermenting) family
- ISWI (imitation switch) family
- CHD (chromodomain, helicase, DNA binding) family
- INO80 (inositol requiring 80) family

Although each family of chromatin remodeler has distinct properties, they do share some basic properties:

- Affinity for the nucleosome
- Domains that provides recognition and binding to histone modifications and proteins
- Catalytic ATPase domain involved in DNA/histone disruption (Helicase)

The focus in this thesis is on the SWI/SNF family and further discussions about chromatin remodeling complexes will be about this family. The SWI/SNF family that was first identified in yeast is a > 1MDa complex consisting of 8 to 14 subunits. Most of its function revolves around one of its two catalytic subunits Brm (Brahma) and Brg1 (Brahma related gene 1). Brm/Brg1 harbors a C-terminal bromodomain that recognizes acetylated histones. Gene promoters with high levels of histone acetylation are often enriched in Brm/Brg1 and RNA polymerase II indicating active transcription.

The subunits of the complex are exchangeable, contributing to the functional specificity of Brm and Brg1. Kadam and Emerson showed that Brg1 preferentially binds to proteins with zinc finger motifs while Brm to proteins with ankyrin repeat motifs [84]. Differences between Brg1 and Brm can also be seen phenotypically. Mice lacking Brm are up to 15 % heavier and show altered cellular proliferation compared to normal mice [85]. While Brg1 null mice are embryonic lethal [86]. Despite specificity differences, functional redundancy has also been observed [87, 88].

1.6 THE IGF FAMILY

The insulin-like growth factor (IGF) family plays key roles in mammalian growth, development and metabolism by regulating cell proliferation, differentiation, survival, migration and glucose metabolism. Components of the IGF system includes three soluble ligands insulin, IGF-1 and IGF-2, three cell surface receptors, insulin receptor, IGF-1R and IGF-2R, six IGF binding proteins 1-6 (IGFBP) and their IGFBP proteases. The insulin receptor is due to alternative splicing present in two isoforms, isoform A (without exon 11) or isoform B. Furthermore IGF-1R and insulin receptors are capable to form hybrid receptors [89, 90].

For the remaining part of this thesis I will mainly focus on the IGF receptors and its ligands IGF-1 and IGF-2.

1.6.1 Insulin-like growth factors

IGFs are potent mitogens regulating growth, development and metabolic processes. Both IGF-1 and IGF-2 are primarily produced in the liver and delivered to target cells through circulation. They exert their function by binding and activating their cognate receptor IGF-1R on the cell surface, thereby initiating a cascade of cytoplasmic signaling events resulting in the activation of numerous genes [91-93]. High levels of IGFs are also correlated with increased cancer risk [94-96]. Cancer cells often sustain high levels of IGF signaling by developing autocrine loops where the cells express both the IGF ligands and receptors [97, 98].

The *IGF-1* gene is located on the long arm of chromosome 12 spanning 9 kb. It contains two promoters and six exons encoding a mature 7.5 kDa IGF-1 peptide of 70 amino acids [99-101]. Production of IGF-1 is stimulated by secreted growth hormones produced by the pituitary gland [102]. Serum levels of IGF-1 vary during lifetime. Fetal serum contains low levels of IGF-1 peaking at 100 ng/ml at full term pregnancy. A second peak is observed during puberty and thereafter decreases with age. IGF-1 concentrations in adults are about 100-200 ng/ml [103].

IGF-2 is a 67 amino acid long peptide showing 62 % sequence similarity to IGF-1. The *IGF-2* gene is located on the short arm of chromosome 11 and contains four promoters P1-P4 of which P2-P4 are maternally imprinted [104]. Loss of imprinting is correlated with cancer [105, 106]. IGF-2 is suggested to have a more prominent role than IGF-1 in embryonic growth [107]. Serum levels of IGF-2 reach 300 ng/ml at term, peaking at 700 ng/ml after birth. Adults have 400 – 600 ng/ml of IGF-2. Despite higher and more stable serum levels of IGF-2 compared to IGF-1, IGF-2 is believed to play a less prominent role in adults. Furthermore, in contrast to IGF-1, IGF-2 production is less sensitive to growth hormones [108].

1.6.2 IGF binding proteins

IGF binding proteins 1-6 (IGFBPs) have a wide repertoire of functions. The most well known function is to bind circulating IGFs. Approximately 98% of circulating IGFs in plasma are bound by IGFBPs of which IGFBP-3 accounts for nearly 80% [109]. By binding to IGFs with higher affinity than IGF-1R they regulate much of the activities of IGFs by sequestering them from IGF-1R, protection from degradation and transportation to target cells.

Besides IGF dependent activities, several studies have indicated that IGFBP can have IGF independent functions. Jones *et al.* showed that IGFBP-1 promoted migration of Chinese hamster ovary cells, independent of IGFs, by binding to integrin α5β1 through its integrin recognition site [110]. Another study demonstrated that IGFBP-3 inhibits TGF-β1 binding to type V TGF-β receptor in mink lung epithelial cells [111]. IGFBP levels are controlled by IGFBP proteases. Proteolytic cleavage of IGFBPs releases IGFs thereby increasing their availability for IGF-1R [112].

1.6.3 Insulin-like growth factor receptor

Of the three receptors in the IGF family, it is IGF-1R that elicits most of the biological effects of IGF-1 and IGF-2. Though both IGF-1R and IGF-2R belongs to the IGF family and is able to bind IGF-2, they differ both structurally and functionally.

IGF-2/mannose-6-phosphate receptor binds IGF-2 at the cell surface. It has no kinase domain or any signaling capabilities. When bound to IGF-2, the receptor-ligand complex is internalized and trafficked through endosomal vesicles to the lysosome where IGF-2 is released and degraded. By doing so, IGF-2R is able to clear the availability of IGF-2 and thereby attenuate its biological activity. IGF-2R also binds proteins tagged with mannose-6-phosphate (M6P), hence its alternative name M6P receptor, in the *trans*-Golgi network. Just like with IGF-2, binding to M6P-tagged proteins results in the trafficking to lysosome for destruction.

1.7 IGF-1R

1.7.1 Structure

The *IGF-1R* gene is located on the long arm of chromosome 15 (chr 15 q26.3) spanning almost 100 kb and consists of 21 exons. The promoter lacks a CCAAT binding element and a TATA box but contains several SP1 and AP2 binding sites [113, 114]. It was recently shown that IGF-1R could auto-regulate its own gene expression [115].

IGF-1R protein (and insulin receptor) is, unlike other RTKs, not a monomer that dimerizes upon ligand binding, but exists as a heterotetramer at all time. IGF-1R constitutes of two α and two β subunits held together with disulfide bonds. The two α subunits makes up the extracellular ligand binding domain, while the β subunits makes up the transmembrane domain, intracellular tyrosine kinase domain and the C-terminal domain. The intracellular domains contain numerous phosphorylation sites important for its signaling capabilities (Figure 2).

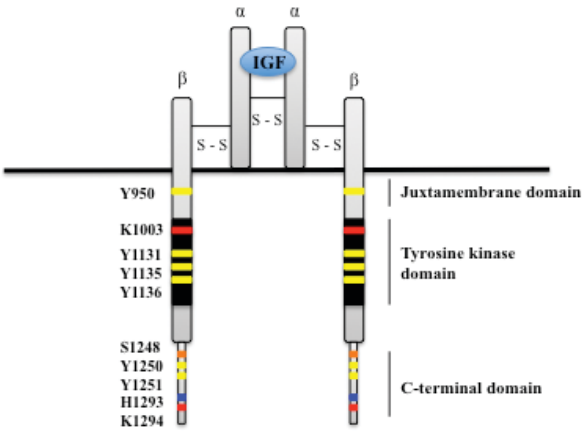

Figure 2: Structure of IGF-1R including important domains and residues. S-S = disulfide bonds, Y = tyrosine, K = lysine, S = serine, H = histidine.

1.7.2 Signal transduction

Signaling through IGF-1R begins when one of its ligands binds the receptor in the extracellular domain. Ligand binding initiates a trans-autophosphorylation event where the three tyrosine residues, Tyr1135, Tyr1131 and Tyr1136 in the activation loop located in the kinase domain become sequentially phosphorylated. This leads to a conformational change of the activation loop increasing the activity of the kinase to maximum level. As a result, the kinase is able to efficiently phosphorylate additional residues, which will function as docking sites for SH2 proteins to transduce the IGF signal via a cascade of cytoplasmic signaling pathways [116, 117].

Numerous residues in the β subunits have been identified to be crucial for IGF-1R function. Tyr950 in the juxtamembrane region is important for the binding of adaptor proteins insulin receptor substrate (IRS) 1-4 and Shc. Lys1003 is the ATP binding site, and together with Tyr950 are important sites for the mitogenic and transformation properties of IGF-1R [116, 118, 119]. Tyr1250, Tyr1251, His1293 and Lys1294 are critical for the anti-apoptotic as well as transformation properties [120-122]. More recently it was shown that Ser1248 residue regulated IGF-1R autophosphorylation and its ability to activate Akt signaling pathway [123].

Since IGF-1R mediates much of its biological functions from the plasma membrane by activating cytoplasmic signaling pathways, I will in the following section further discuss two major signaling pathways that are activated by IGF-1R, namely MAPK/Erk and PI3K/Akt signaling pathways. However I would like to emphasize that these two pathways, as mention previously, are activated by other receptors as well. But for the sake of this thesis I will keep the discussion about the two pathways in relation to IGF-1R.

1.7.3 PI3K/Akt signaling

PI3K/Akt signaling pathway mediates much of the biological effects of IGF-1R (Figure 3). The signaling pathway is triggered by the activation of IGF-1R. Following activation, IRS adaptor proteins are recruited to phosphorylated Tyr950 of IGF-1R. This result in the IRS proteins being phosphorylated themselves at tyrosine residues. Two of these residues, Tyr608 and Tyr632 (IRS-1) are key for efficient recruitment of p85 regulatory domain and activation of p110 catalytic domain of PI3K [124]. Fully activated PI3K converts phosphatidylinositol (3, 4)-bis phosphate (PIP2) lipids to phosphatidylinositol (3, 4, 5)-tris phosphate (PIP3) second messengers. Akt binds PIP3 at the plasma membrane, which in turn becomes available for PDK1 (phosphoinositide-dependent kinase-1), a serine/threonine kinase. PDK1 phosphorylate Akt at Thr308 to partially activate Akt [125]. Complete activation of Akt is dependent on the phosphorylation of Ser473 by mTOR (mammalian target of rapamycin) [126] or by DNA-PK (DNA dependent protein kinase) [127].

Activated Akt phosphorylates and regulates the activity of numerous proteins linked to apoptosis, proliferation and metabolism. These include the pro-apoptotic FOXO proteins. Phosphorylation of FOXO proteins by Akt inhibits their function, thereby preventing cells to undergo apoptosis [128]. Another important target protein is the protein complex mTORC1 (mammalian target of rapamycin complex 1). mTORC1 stimulates protein synthesis but its activity is inhibited by PRAS40 (proline-rich Akt substrate of 40 kDa). Phosphorylation of PRAS40 by Akt, frees mTORC1 from its inhibition. This renders mTORC1 able to activate its substrates eIF4E (eukaryotic translational initiation factor 4E) and S6K1 (ribosomal protein S6 kinase beta-1) resulting in protein synthesis [129, 130]. Akt has also been shown to regulate glycogen metabolism by phosphorylating and inactivating GSK3 [124, 131]. Inactivation of GSK3 releases GLUT4 (glucose transporter type 4) from its intracellular vesicles and becomes incorporated into the plasma membrane where it transports glucose.

Inactivation of PI3K/Akt signaling pathway is mediated by phosphatases that dephosporylates key proteins of the pathway. Andjelkovic *et al.* showed that PPA2 (protein phosphatase 2A) dephosphorylated Akt Thr308 [132]. The second phosphorylation site S473, which resulted in full activation of Akt, was shown to be dephosphorylated by PHLPP (plecktstrin homology domain leucine-rich repeat protein phosphatase) [133]. Inactivation of other components of the pathway includes PIP3 which is converted back to PIP2 by PTEN (phosphatase and tensin homolog) [134]. Furthermore, inactivating phosphorylation of IRS has also been demonstrated. While tyrosine phosphorylation of IRS results in the recruitment and activation of PI3K, phosphorylation of Serine312 and Serine798 attenuates the signal [135, 136].

1.7.4 MAPK/Erk signaling

Another major signaling pathway that mediates the biological effects of IGF-1R is the MAPK/Erk signaling pathway (Figure 3). MAPK/Erk signaling involves the activation of the G protein Ras followed by a cascade of phosphorylation events mediated by three tiers of kinases.

In contrast to PI3K/Akt signaling, which utilizes IRS adapter proteins, MAPK/Erk signaling involves the recruitment of Shc adapter proteins to phosphorylated Tyr950. Shc in turn becomes tyrosine phosphorylated, thus bringing the cytosolic protein Grb2 that is complexed with SOS (Son of Sevenless) to the plasma membrane where Ras is located. SOS is a guanine exchange factor that activates Ras protein. Ras is a GTPase that belongs to the family of G proteins. SOS activates Ras by exchanging GDP to GTP. Ras-GTP is then able to activate the first tier of kinases in the pathway, Raf-1 (c-Raf) kinases. In its inactive form, the N-terminal domain of Raf-1 adopts a conformation that blocks its own kinase domain. Ras binding causes a conformational change of the N-terminal, which exposes the kinase domain thereby rendering Raf-1 active [137]. This is followed by the activation of second tier of kinases MEK1/2. MEK1/2 are dual specificity kinases as they can phosphorylate serine/threonine residues as well as tyrosine residues. Activation of MEK1/2 by Raf-1 is achieved when two serine residues in the activation loop, Ser218 and Ser222 (MEK1) become phosphorylated [138, 139]. The third tier consists of the Erk proteins. Erk proteins are highly conserved proteins consisting of Erk1 and Erk2. MEK1/2 phosphorylate Erk proteins at Thr and Tyr residues within the Thr-Xaa-Tyr motif leading to their activation and nuclear translocation [140]. Nuclear Erk phosphorylates numerous transcription factors such as p53, Myc, c-Fos, Elk1, Ets1/2 and c-Jun, thereby regulating the expression of a vast variety of genes.

The MAPK/Erk signaling pathway is regulated at several levels. MEK proteins can undergo phosphorylation/dephosphorylation in addition to the phosphorylations at Ser218 and Ser222. Phosphorylation of MEK1 Ser298 by PAK1 (p21 activated kinase) has a positive effect on MEK1 [141] while negative feedback phosphorylation at Thr292 by Erk proteins inactivates it [142]. Dephosphorylation of Ser218 and Ser222 by PP2A also inactivates MEK [143]. Inactivation of Erk involves the dephosphorylation of the Thr and Tyr residues in the Thr-Xaa-Tyr motif. This can be achieved by the Ser/Thr phosphatase PPA2 [144], Protein Tyr phosphatase PTP-SL [145] or dual specificity phosphatases termed MAPK phosphatases [146].

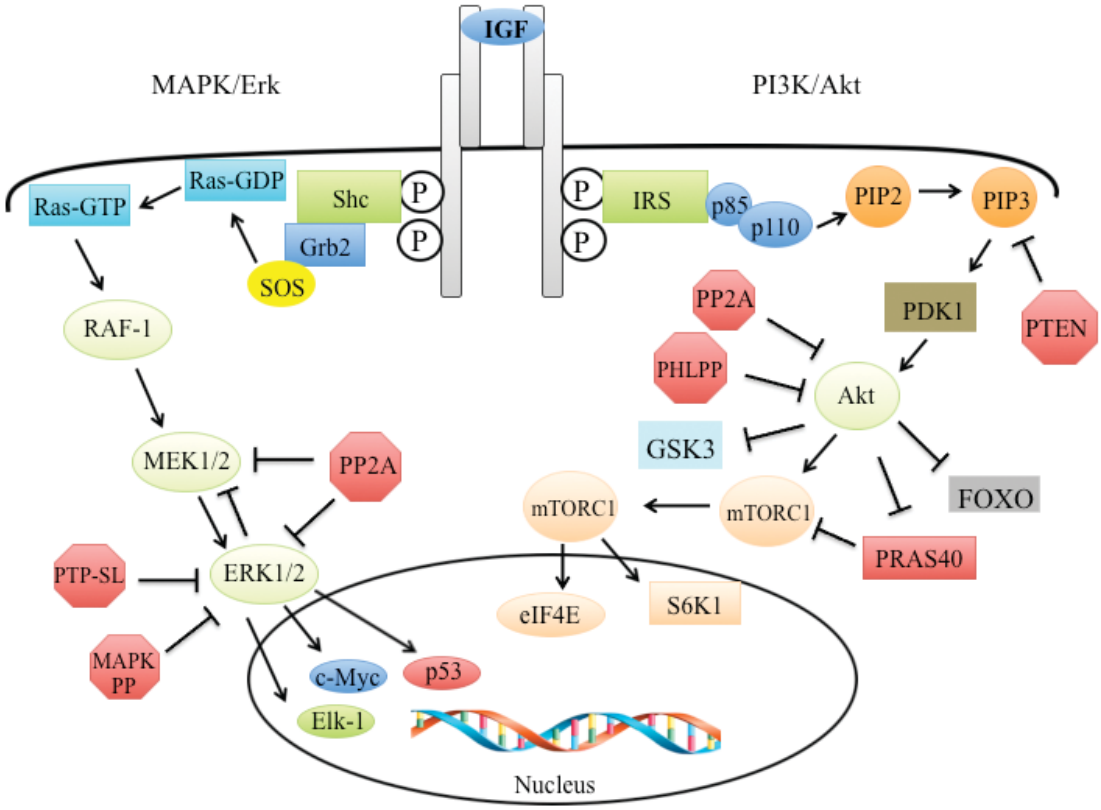

Figure 3: Overview of the MAPK and PI3K signaling pathways upon IGF-1R activation.

1.8 IGF-1R AND CANCER

Given the plethora of genes that are regulated by PI3K and MAPK signaling pathways, it is not surprising that IGF-1R plays key roles in physiology and disease. Early studies on IGF-1R function concluded that the receptor is highly active during prenatal stages as a key protein for proper tissue development. Measurement of mRNA levels of IGF-1R from various stages of rat embryogenesis showed that the levels peaked during prenatal stages and rapidly declined thereafter [147]. The importance of IGF-1R during embryogenesis was further highlighted in a study with IGF-1R null mice. Mice lacking IGF-1R were 45 % of the size of wild type mice and died shortly after birth due to organ hypoplasia and defects in the central nervous system [148].

The importance of IGF-1R in malignant transformation was highlighted in a study from Renato Baserga's laboratory [149]. Mouse embryonic fibroblasts cells with a targeted disruption of the *IGF-1R* gene, called R- cells, had a markedly slower growth rate compared to the parental wild type cells. R- cells also lacked the ability to grow under anchorage-independent conditions as well as lacking the ability to form colonies while growing as monolayer. Introduction of the SV40 large T antigen oncogene into the parental cell line induced cell transformation. However, transformation could not be induced in R- cells, unless wild type IGF-1R was reintroduced, thus showing the importance of IGF-1R in malignant transformation.

It is now well established that IGF-1R plays a key role in malignant transformation. Numerous studies have provided evidence that IGF-1R regulates cancer cell proliferation, survival, invasion and metastasis. Elevated levels of IGF-1R are correlated with poor prognosis. This has prompted researchers to view IGF-1R as a possible drug target [150]. Antibody based drugs that blocks IGF-1/IGF-1R interaction or small chemical compounds, which inhibit the tyrosine kinase domain, are currently the main strategies for inhibiting IGF-1R [151]. However, most of the drugs currently in development have failed in phase III clinical trials [150]. Successful drug development requires a better knowledge about IGF-1R function. Although the importance of IGF-1R in malignant transformation is undisputed, the molecular mechanisms by which IGF-1R regulates its downstream targets are still an area to be explored.

It was recently demonstrated from our laboratory that IGF-1R is subjected to modification with small ubiquitin-related modifier (SUMO) proteins in cancer cells. SUMO modification of IGF-1R resulted in its nuclear accumulation. These novel findings add another level of complexity of IGF-1R function and have to be considered when developing drugs against IGF-1R.

1.9 SUMOYLATION

SUMOylation is a reversible process that involves the attachment of a SUMO protein to lysine residues of the target protein. The first protein that was identified to be SUMO modified was RanGAP1 [152, 153]. SUMOylated RanGAP1 is targeted to the nuclear pore complex where it hydrolyzes Ran-GTP. Since these original studies, a vast variety of proteins have been identified to be SUMO targets. SUMOylation regulates protein stability [154], protein-protein interactions [155] and subcellular localization [156], thereby impacting nearly all processes in the cell. The SUMOylation process is energy dependent similar to the ubiquitination process and involves three classes of enzymes, E1, E2 and E3.

1.9.1 SUMO proteins

There are three main SUMO proteins in mammals. A fourth SUMO protein has been described, though its function is still unclear. They belong to the family of ubiquitin-like proteins (Ubls) [157]. Ubls are characterized by the ubiquitin fold and their C-terminal Gly-Gly motif that is cleaved during the maturation process. The 20 amino acids at the N-terminal function as an acceptor for other SUMO proteins to form SUMO chains similar to ubiquitin chains. SUMO proteins are divided into two families, SUMO1 and SUMO2/3. SUMO1 protein show 47% sequence similarity with SUMO2, while SUMO2 and SUMO3 show 97% sequence similarity. Due to the high similarity between SUMO2 and SUMO3 they are often referred to as SUMO2/3. The majority of SUMO2/3 proteins are found in its unconjugated form and expressed 10 times more than SUMO1 [158].

The importance of SUMO proteins in physiology and disease is illustrated in SUMO deletion mutants of *Schizosaccharomyces pombe* [159] and the filamentous fungus *Aspergillus nidalus* [160], which display severe growth defects. In mammals, SUMO1 haploinsufficiency cause cleft lip and palate [161]. There is also extensive data supporting involvement of SUMOylation in tumorigenesis [162-164].

1.9.2 The SUMOylation process

SUMO attachment to its substrate is energy dependent. The SUMOylation process is highly similar to ubiquitination requiring three classes of enzymes, the SUMO E1 activating enzyme, E2 conjugating enzyme and E3 ligase. The SUMO E1 enzyme is composed of two subunits, SUMO-activating enzyme subunit 1 (SAE1) and SUMO-activating enzyme subunit 2 (SAE2). E1 enzyme activates SUMO at its C-terminal by adenylation followed by the formation of a thioester bond between SUMO and SAE2 subunit of E1 enzyme. This causes the single E2 conjugating enzyme, Ubc9, to interact with SAE2. The activated SUMO is transferred to Ubc9 through the formation of a new thioester bond. Many SUMO targets harbors a consensus site for Ubc9 interaction and studies have shown that Ubc9 has the ability to SUMOylate target proteins in absence of E3 ligases *in vitro*. This has lead to the questioning of whether E3 ligases are needed. However, the efficiency of SUMOylation is much lower in absence of an E3 ligase [165]. There are three families of E3 ligases 1) RanBP2, a nuclear pore complex protein discussed earlier, 2) protein inhibitor of activated STAT (PIAS), 3) Polycomb protein 2 (Pc2). The E3 ligases catalyze the transfer of the SUMO protein from E2 enzyme to target lysine residue of the substrate.

1.9.3 SUMO proteases

As SUMOylation is a reversible process, enzymes that catalyze the removal of SUMO proteins from their target are needed. The six sentrin specific proteases (SENP1-3, 5-7), belonging to the SENP family, cleaves between the terminal Gly reside of SUMO and the Lys residue of the SUMOylated target. Members of the SENP family were thought to be the only SUMO proteases to exist until recently when three additional proteases were identified, desumoylating isopeptidase 1/2 (DESI1/2) and ubiquitin-specific protease like 1 (USPL1) [166-168]. Target specificity of SUMO proteases is partly determined by their subcellular localization. The majority of the SUMO proteases are located in the nucleus or specific nuclear compartments. Furthermore, most proteases show higher preferences towards SUMO2/3 proteins. The mechanism governing this preference is currently unknown. In addition to removing SUMO proteins, SUMO proteases also participate in the maturation process of SUMO by cleaving the C-terminal Gly-Gly motif of SUMO making SUMO proteins ready for activation.

1.9.4 SUMO targets

Given that SUMO enzymes and SUMO proteases are highly enriched in the nucleus it is easy to believe that SUMOylation is a nuclear event. Also the fact that many SUMO target proteins exert their functions in the nucleus, such as transcription factors, DNA repair

proteins and chromatin remodeling factors further ingrain this view. However, proteins in non-nuclear compartments are SUMOylated as well. RanGAP1, which was mentioned earlier, is one such protein. Though RanGAP1 plays a key role in nucleo-cytoplasmic trafficking and thereby regulating nuclear events, RanGAP1 *per se* is not a nuclear protein. The cytoplasmic tumor suppressor PTEN has been shown to be a SUMO target. SUMOylated PTEN is targeted to the plasma membrane and inactivates PI3K signaling [169]. Proteomic studies have suggested that SUMOylation also occurs co-translationally upon stress [170]. If this is the case, sorted proteins that normally would be hidden or masked from the SUMOylation machinery could potentially become SUMOylated as they are being translated.

A more recent group of proteins, the cell surface receptor kinases has been demonstrated to be SUMO targets. In a study from 2008, Kang *et al.* showed that the cell surface serine/threonine kinase receptor TGF-β1 receptor was SUMO modified resulting in increased Smad3 signaling [171]. Research from our own laboratory has provided evidence that the IGF-1R is SUMOylated with SUMO1 at the nuclear periphery leading to its nuclear accumulation [172]. ErbB4 receptor tyrosine kinase has also been shown to be a SUMO target [173]. The cleaved intracellular domain of ErbB4 was demonstrated to be SUMOylated by members of the PIAS family of E3 ligases. This resulted in its sequestration into nuclear bodies.

These findings demonstrate two things, 1) cell surface receptor kinases, which are mainly associated with phosphorylation, are also SUMO targets 2) receptor tyrosine kinases, whose primary function is to signal from the cell surface into the nucleus, can be localized in the nucleus themselves. Though earlier studies have demonstrated nuclear localization of receptor tyrosine kinases, their functions and modes of nuclear transportation are still areas to be further explored.

1.10 NUCLEAR RTKS

Traditionally, RTKs connects extracellular stimuli to nuclear events by initiating a cascade of cytoplasmic signaling pathways that modulates gene expression. Activated RTKs are internalized and are then either targeted for destruction or recycled back to the plasma membrane. This view has, however, been broadened as several studies have provided evidence that RTKs and their ligands can exist in the cell nucleus. Studies from the mid 80s demonstrated that the growth factors EGF and PDGF together with were speculated to be their cognate receptors was found in the nucleus associated with chromatin in several human carcinoma and fibroblast cell lines [174]. Since then, many other RTKs have been found in the nucleus including other members of the EGFR family [175-177], NGFR [178], FGFR [179, 180], insulin receptor [181] IGF-1R [172, 182, 183], VEGFR [184], c-Met receptor [185] and several others.

1.10.1 Functions

As the list of RTKs found in the nucleus is growing longer, more focus is being put on trying to understand their nuclear functions. Most studies regarding nuclear RTKs has been done on

EGFR, thus it is the prototypical nuclear RTK. Given the fact that the original study from the mid 80s demonstrated that EGFR was associated with chromatin, much focus on nuclear EGFR has been on its transcriptional activities. Lin *et al*. showed that nuclear full-length EGFR was bound to AT-rich sequences in the *cyclin D1* promoter and induced its expression in cancer cells [186]. Expression of *cyclin D1* by nuclear EGFR was later shown to be in collaboration with RNA Helicase A [187]. Nuclear EGFR also form complexes with several transcription factors such as STAT3, STAT5, and E2F1 to induce expression of *iNOS*, *AURORA A* and *B-MYB* [188-190]. Phosphorylation of proliferating cell nuclear antigen (PCNA) protein at Tyr211 by nuclear EGFR stabilized PCNA thereby increasing cell proliferation [191]. In addition to full-length EGFR, ErbB4 (member of EGFR family) can be cleaved that produces a soluble fragment of the intracellular domain (ICD). ErbB4 ICD translocate into the nucleus to participate in transcriptional activities [192].

Another nuclear RTK that has been relatively well studied is the FGFR. Nuclear FGFR is correlated with highly proliferative cell states [180, 193]. In addition to full-length nuclear FGFR, the intracellular domain of FGFR1 can be cleaved and translocate into the nucleus where it promotes cell migration in breast cancer cells [194]. In human medulloblastoma cells nuclear FGFR1 was demonstrated to bind the histone acetyl transferase creb-binding protein (CBP) in nuclear speckle domains. Binding to CBP displaced ribosomal S6 kinase from CBP thereby relieving CBP from its inhibition. Active CBP recruited RNA polymerase II and acetylated histones at gene promoters [195].

These and other studies demonstrate that RTKs are more than just activators of cytoplasmic signaling pathways, they can have a more direct role in the nucleus regarding gene regulation.

1.10.2 Mechanism for nuclear translocation

In order to fully appreciate the fact that RTKs can transport into the nucleus, it is of highest interest to understand the mechanism for this transportation. Our current knowledge about EGFR transportation into the nucleus is that it's dependent on endocytosis [196, 197]. The steps following endocytosis is not completely clear. A key issue that needs to be resolved is how a hydrophobic transmembrane receptor can escape its membrane environment. The answer to this question was proposed by Liao and Carpenter which suggested that EGFR utilizes the endoplasmatic reticulum associated protein degradation (ERAD) pathway to translocate into the nucleus [198]. The ERAD pathway is dependent on the bidirectional ER translocon sec61 and is utilized by the cell to translocate misfolded proteins in the ER into the cytosol for degradation. Certain viruses also enter through the ERAD pathway. Liao and Carpenter suggested that endocytosed EGFR is translocated into the ER via sec61 in a ligand dependent manner. EGFR in the ER was shown to be transported into the cytosol where it complexes with the chaperone HSP70 and enters the nucleus. How EGFR escape degradation when it is transported back into the cytosol is unknown. Transport through the ERAD pathway was suggested to be a potential mechanism for EGFR to be extracted from its membrane compartment. It was hypothesized that HSP70 binds the transmembrane region of EGFR in the cytosol thereby protecting its hydrophobic region. This study, though novel,

does not provide evidence whether EGFR/HSP70 complex is located within an endosomal vesicle. Other studies have however provided evidence that EGFR is membrane bound during its transportation into the nucleus. Giri *et al.* demonstrated that nuclear ErbB2 was associated with early endosome antigen 1 (EEA1) [196]. Furthermore, cells treated with digitonin, which permeabilizes the plasma membrane and releases soluble cytosolic proteins, did not impair nuclear EGFR and ErbB2 transportation [199]. These studies strongly suggest that EGFR is membrane bound as it is being transported into the nucleus. Whether the ERAD pathway is a general mechanism for nuclear entry utilized by all members of the EGFR family is so far unknown. What is common for all members of the EGFR family is that they harbor a NLS in their juxtamembrane region [200] and nuclear entry is importin-α/β dependent [197]. For ErbB2, it was also demonstrated to complex with the nuclear pore complex protein RanBP2 [196].

Nuclear entry of cell surface FGFR also seems to be dependent on translocation into the ER via sec61. Though in contrast to members of EGFR, FGFR1 is transported into the nucleus as a soluble, non-membrane bound protein since digitonin treatment diminished FGFR1 levels in the nucleus [199]. The fact that FGFR1 could be transported as a soluble protein was attributed to its unconventional transmembrane domain, which consists of short stretches of hydrophobic amino acids interrupted by hydrophilic amino acids [201]. Nuclear entry of FGFR is dependent on importin-β [202]. Though none of the members of FGFR family harbor any known NLS, some of its ligands, such as FGF2 do [203]. This means that the ligands not only activates the receptor but also directs it to the nucleus.

1.10.3 Nuclear IGF-1R

First evidence of IGF-1R in the nucleus was in hamster kidneys [183]. Treatment of hamster kidneys with the synthetic estrogen diethylstilbestrol caused an up-regulation of nuclear IGF-1R. But it was not until 15 years later where studies showed that IGF-1 treated cancer cells caused nuclear translocation of IGF-1R in a SUMOylation dependent manner [172, 182]. IGF-1R is SUMOylated at residues Lys1025, Lys1100 and Lys1120 with SUMO1. A mutant IGF-1R where all three lysine residues were mutated (TSM IGF-1R) abolished SUMOylation and inhibited nuclear accumulation.

Like other RTKs, nuclear IGF-1R is correlated with highly proliferative cells [204]. Functionally, nuclear IGF-1R has been shown to bind enhancer elements [172] as well as its own gene to auto-regulate its expression [115] in cancer cells. In corneal epithelial cells IGF-1R/insulin receptor hybrids associated with genomic DNA [205]. In these studies full length IGF-1R accumulated in the nucleus. In orbital fibroblasts from Grave's disease, ADAM17 protease cleaves IGF-1R and the cleaved fragment translocate into the nucleus [206]. Interestingly though is that here it is the extracellular domain of IGF-1R that is targeted to the nucleus instead of the intracellular domain like other RTKs. The mechanism that accounts for this is unknown.

Much of our current knowledge about IGF-1R function comes from studies with the premise that IGF-1R is a plasma membrane exclusive receptor. To fully appreciate the role of IGF-1R in physiology and disease our view of IGF-1R needs to be broadened.

2 AIMS OF THESIS

The overall aims of this thesis were to investigate the functions of nuclear IGF-1R and to elucidate the mechanism that mediates nuclear transportation of IGF-1R in cancer.

The specific aims of this thesis were:

- To investigate the role of nuclear IGF-1R as a transcriptional activator for Wnt target genes.
- To elucidate the transportation mechanism that mediates nuclear IGF-1R.
- To investigate the role of nuclear IGF-1R as an epigenetic regulator of gene transcription.

3 RESULTS AND DISCUSSION

3.1 PAPER I

Nuclear IGF1R is a transcriptional co-activator of LEF1/TCF

The discovery of nuclear IGF-1R revealed additional functions of IGF-1R that was previously unknown. Apart from its classical role at the cell surface as an activator of cytoplasmic signaling pathways upon extracellular stimuli, nuclear IGF-1R could potentially take a more direct role in the nucleus to exert its biological functions. Since our original discovery in 2010 [172] demonstrating that nuclear IGF-1R was associated with genomic DNA, we were further interested to explore its role in the nucleus with an emphasis on gene regulation.

In this study we demonstrate that nuclear IGF-1R is associated with components of the Wnt signaling pathway and enhance expression of Wnt target genes. We identified association between IGF-1R and β-catenin at the plasma membrane and in the nucleus of DFB (melanoma), HeLa (cervical cancer) and H1299 (non-small cell lung carcinoma) cancer cell lines. Furthermore, nuclear IGF-1R was associated with LEF-1 transcription factor in a ligand dependent manner. Previously it was shown that IGF-1 stimulation caused β-catenin and IRS1 to accumulate in the nucleus and activation of TCF/LEF-1 [207]. β-catenin activates LEF-1 by binding to the N-terminal region of LEF-1 and thereby displacing the inhibitor Groucho [48, 49]. We removed the N-terminal β-catenin binding domain of LEF-1 but did not see a diminished IGF-1R binding to LEF-1 suggesting that IGF-1R binds LEF-1 independently of β-catenin. As for IRS1 we speculated whether it could mediate nuclear translocation of IGF-1R and/or function as a scaffold for IGF-1R/LEF-1. To address this question we utilized mouse fibroblasts derived from IGF-1R null mice (R- cells) which were stably transfected with wt IGF-1R (R+) or an IGF-1R with a mutation in the IRS1 binding site (R-ΔIRS1). Our data show that nuclear translocation of IGF-1R and IGF-1R/LEF-1 interaction is IRS1 independent.

We next investigated whether Wnt target genes were under the control of nuclear IGF-1R. In order to distinguish the nuclear activities from the non-nuclear activities of IGF-1R we took use of an IGF-1R mutant, which has its three SUMOylation sites mutated (tsm IGF-1R). Tsm IGF-1R is unable to translocate into the nucleus but retains its signaling capabilities [172]. We specifically investigated the effect of nuclear IGF-1R on cyclin D1 and axin2. Luciferase assays were performed with *cyclin D1* and *AXIN2* promoter constructs fused to a luciferase reporter gene. Transiently transfected H1299 cells with wt or tsm IGF-1R together with the promoter constructs show that wt IGF-1R enhances the activity of both promoters with 17% and 22% respectively compared to the control. On the other hand promoter activity was decreased in tsm IGF-1R cells by 7% and 20% respectively. The protein levels of cyclin D1 and axin2 were also investigated yielding similar results as the luciferase assay. Since it was previously demonstrated that IGF-1R could bind genomic DNA we speculated that increase in cyclin D1 and axin2 levels could be explained by the binding of IGF-1R to their respective

promoters. As a prof of concept we performed chromatin immunoprecipitation to investigate enrichment of IGF-1R to the *cyclin D1* promoter. Enrichment of endogenous IGF-1R to *cyclin D1* promoter in DFB and HeLa cancer cells could clearly be detected. H1299 cells transiently transfected with wt and tsm IGF-1R showed a 120-fold enrichment of wt IGF-1R to the *cyclin D1* promoter but not of tsm IGF-1R. Taken together, this study demonstrate that IGF-1R is more than a classical cell surface receptor, it can translocate into the nucleus to regulate the expression of cyclin D1 and axin2 by associating with LEF-1 transcription factor and the *cyclin D1* promoter.

3.2 PAPER II

Nuclear translocation of IGF-1R via p150^{Glued} and an importin-β/RanBP2-dependent pathway in cancer cells

The aim of this study was to elucidate the transportation mechanism for nuclear IGF-1R. We present a mechanism that involves the retrograde transport of IGF-1R by the dynactin complex, the action of importin proteins and the E3 ligase activity of RanBP2.

Firstly, we show that IGF-1R is associated with EEA1 in both the cytoplasm and the nucleus, similar to the EGFR, in H1299 cells. This suggests that IGF-1R is membrane bound during the transport. Inward transport of proteins along microtubules is dependent on retrograde transport machinery involving the dynactin complex [208]. The largest subunit of dynactin, p150^{Glued} connects the transported protein to microtubules and the motor protein dynein. To verify that IGF-1R takes this route into the nucleus we initially treated cells with the microtubule disruptor colchicine and looked for nuclear IGF-1R. Colchicine treatment reduced the amount of nuclear IGF-1R. Next we performed *in situ* proximity ligation assay (PLA) to investigate if IGF-1R and p150^{Glued} were interacting partners. Our results show that this indeed is the case. Interactions are detected in the cytoplasm and around the nucleus. Knock down of p150^{Glued} with siRNA diminished cells of nuclear IGF-1R by almost 50%. We also overexpressed dynamitin, the smallest subunit of dynactin. Overexpression of dynamitin is known to disrupt the dynactin complex and is often used to study dynactin mediated processes. Similar to the results when p150^{Glued} was knocked down, overexpression of dynamitin diminished cells of nuclear IGF-1R with almost 50%.

As previously mentioned, nuclear import of EGFR and FGFR is dependent on importin-β. PLA and co-immunoprecipitation experiments show that IGF-1R is associated with importin-β. This association was disrupted when dynamitin was overexpressed indicating that dynamitin is upstream of importin-β. Knock down of importin-β reduced the levels of nuclear IGF-1R with over 50%. Passage through the NPC is also dependent on Ran GTPase. To verify this, we transfected cells with a Ran mutant (RanQ69L) that is unable to hydrolyze GTP to GDP. In similar fashion to importin-β siRNA, RanQ69L overexpression also reduced nuclear IGF-1R levels.

Next we investigated the possible role of RanBP2. Giri *et al.* had shown that transport of ErbB2 into the nucleus was dependent on RanBP2 [196]. With PLA and co-

immunoprecipitation we concluded that RanBP2 is an interacting partner for IGF-1R. Furthermore, the interaction is dependent on IGF-1R SUMOylation and importin-β. In all co-immunoprecipitation experiments conducted so far, N-Ethylmaleimide (NEM) had been added to the lysis buffer. NEM is a compound that inhibits deSUMOylation. Removal of NEM from the lysis buffer disrupted IGF-1R/RanBP2 interaction. Knocking down importin-β or overexpressing an importin-β mutant, which is unable to bind RanBP2, diminished both IGF-1R/RanBP2 interaction and nuclear IGF-1R. This suggests that importin-β is upstream of RanBP2. Knock down of RanBP2 decreased the levels of nuclear IGF-1R by 80%, which further demonstrates the involvement of RanBP2. We continued our study on RanBP2 by focusing on its SUMO E3 ligase activity. Since nuclear translocation of IGF-1R is SUMO dependent and that RanBP2 is located on the cytoplasmic face of the NPC, we speculated that RanBP2 might SUMOylate IGF-1R. To address this question we utilized a DNA construct encoding the SUMO E3 domain of RanBP2. The reason for why we used this construct instead of full-length RanBP2 is because full-length RanBP2 is difficult to transfect, most likely due to its large size. Overexpression of the SUMO E3 ligase enhanced IGF-1R SUMOylation with 55%. However, this did not result in an increased amount of nuclear IGF-1R. This is probably because the localization of the SUMO E3 ligase is spread throughout the cytoplasm and not anchored to the NPC as the full-length RanBP2. Furthermore, we show that SUMOylation of IGF-1R by RanBP2 stabilizes IGF-1R protein levels. These data suggest that one function of RanBP2 is to SUMOylate IGF-1R, thereby stabilizing IGF-1R and enables it for nuclear import. Taken together, in this study we propose a model, which explains the transportation mechanism by which nuclear IGF-1R is mediated. The model shows similarities with nuclear EGFR transportation, as it is dependent on EEA1, importin- β and RanBP2. However, whether nuclear IGF-1R utilizes the ERAD pathway to enter the nucleus still needs to be explored. Furthermore, as IGF-1R do not harbor any known NLS it would be interesting to identify possible adaptor proteins, which IGF-1R might be dependent on for its nuclear transportation.

3.3 PAPER III

Nuclear IGF-1R phosphorylates histone H3Y41 and induces SNAI2 expression via Brg1 chromatin remodeling protein

Having previously demonstrated that nuclear IGF-1R could take a more direct role to regulate gene transcription (paper I), we were interested to further explore this role by investigating its involvement in chromatin remodeling and epigenetics. In an effort to identify binding partners for nuclear IGF-1R we performed mass spectrometry on immunoprecipitated IGF-1R from nuclear extracts and identified histone H3. After our finding, a study from Valentine Macaulay's laboratory showed that nuclear IGF-1R was associated with RNA polymerase II and histone H3 [182]. Though no functional studies were conducted, this study clearly supported the idea of nuclear IGF-1R taking a more direct role in gene regulation.

In this study we confirm the interaction between IGF-1R and histone H3 in HeLa cells with co-immunoprecipitation and PLA. In IGF-1R deficient SKUT-1 cells, this interaction was

absent. SUMOylation and nuclear translocation of IGF-1R is phosphorylation dependent [172]. To verify that the interaction between IGF-1R and histone H3 is phosphorylation dependent, we treated HeLa cells with the IGF-1R tyrosine kinase inhibitor NVP-AEW541 for 24 hours. Our results show a reduced IGF-1R tyrosine phosphorylation and IGF-1R/histone H3 interaction.

Next we investigated the role of this interaction. Histone proteins are highly modified by a vast variety of modifications and phosphorylation is one of the more prominent modifications. However, the majority of histone phosphorylation occurs on serine and threonine residues. It was not until 2009 that several groups reported tyrosine phosphorylation on histone proteins [74, 209-212]. Given the fact that IGF-1R harbors a tyrosine kinase domain we investigated the possibility that nuclear IGF-1R might phosphorylate histone H3. Since it was previously shown that Tyr41 (H3Y41) was phosphorylated by Jak2 to induce expression of *lmo2* oncogene, we specifically focused on Tyr41 [74]. We transiently transfected HeLa cells with wt and tsm IGF1R. Blotting with an antibody that specifically recognizes phosphorylated H3Y41 showed a 5-fold increase in Tyr41 phosphorylation by wt IGF-1R compared to mock and a 2.7-fold increase in tsm. The increase in tsm could be due to signaling. In order to conclude that IGF-1R is the direct kinase, we performed an *in vitro* kinase assay using recombinant histone H3 and recombinant IGF-1R (intracellular part). In absence of IGF-1R, no tyrosine phosphorylation could be detected. Addition of recombinant IGF-1R caused a clear tyrosine phosphorylation on histone H3 suggesting that IGF-1R directly phosphorylates histone H3.

Histone modifications and chromatin remodeling are often interlinked during transcriptional events. Chromatin remodeling factors bind to chromatin through histone modifications. Having demonstrated that IGF-1R phosphorylates histone H3, we hypothesized the possibility for a chromatin remodeling factor to bind phosphorylated histone H3. It was previously demonstrated that Brg1/Brm bound histone H3 in a region close to Tyr41 [213]. Therefore biotinylated histone H3 peptides (amino acids 32-56) that were either phosphorylated or not phosphorylated at Tyr41 were utilized to investigate if the chromatin remodeling protein Brg1 binds to any of the peptides. Incubation of the peptides with HeLa cell extracts show equal binding of Brg1 to both peptides. To test this in a cellular context, wt and mutant H3 (Y41F) constructs were generated and transfected into HeLa cells followed by co-immunoprecipitation with a Brg1 antibody. In this context Brg1 has a higher affinity for wt H3 compared to H3Y41F mutant. The different results between the two experiments suggest two things 1) Brg1 binding to histone H3 is not dependent on Tyr41 phosphorylation 2) Phosphorylation of Tyr41 functions as a stabilizing modification. This is based by the fact that Brg1 does not harbor any SH2/PTB domains. Also, histone modifications rarely occur in isolation. In most cases (if not all) there is extensive crosstalk among modifications. Though we do not provide any evidence for this in this study, it is highly possible that Tyr41 phosphorylation result in a second modification of a nearby amino acid, e.g. acetylation, which is recognized by Brg1 through its bromodomain.

Finally we show that *SNAI2* is a target gene for nuclear IGF-1R and histone H3Y41 phosphorylation. HeLa cells transfected with wt and tsm IGF-1R increased *SNAI2* gene expression with 44% by wt IGF-1R compared to mock while tsm did not. Transfection of H3 plasmids and treatment with IGF-1 ligand for 30 min show that IGF-1 treatment increases *SNAI2* gene expression for all transfections. But wt H3 increased *SNAI2* expression the most (>6-fold) compared to 3.8-fold for H3Y41F mutant. We also show that both IGF-1R and Brg1 are enriched at the *SNAI2* promoter. Taken together our data demonstrate that nuclear IGF-1R binds and phosphorylate histone H3Y41 to induce *SNAI2* expression. This is the first study to show that histones are a direct substrate for a RTK kinase domain. We suggest that the histone H3Y41 phosphorylation stabilizes the association between Brg1 and chromatin to allow chromatin remodeling. However, as Brg1 doesn't harbor any SH2/PTB domains, it is possible that H3Y41 phosphorylation might induce a second modification of a nearby residue, such as acetylation, that is recognized by Brg1 via its bromodomain. This however still remains to be determined.

4 CONCLUSION

The work presented in this thesis provides a new perspective of IGF-1R. The elucidation of IGF-1R nuclear functions and trafficking mechanism reveals that IGF-1R is more than a plasma membrane receptor that activates cytoplasmic signaling pathways. IGF-1R has a more direct and active role when it comes to regulating its target genes. The results in this thesis can be summarized as follows:

I. Nuclear IGF-1R associates with β-catenin and LEF-1 in several cancer cell lines. Association with LEF-1 is independent of IRS1 and β-catenin but dependent on IGF-1 stimulation. Nuclear IGF-1R is enriched at the *cyclin D1* promoter and increase cyclin D1 and axin2 protein levels.

II. Trafficking to the nucleus is dependent on the protein complex dynactin, microtubules, importin-β and RanBP2. SUMOylation of IGF-1R by RanBP2 stabilizes IGF-1R and passage into the nucleus is dependent on Ran GTPase. Disruption of any of these components impairs nuclear translocation of IGF-1R.

III. Nuclear IGF-1R binds and phosphorylates histone H3 at Tyr41. This phosphorylation stabilizes Brg1 association with chromatin and increase *SNAI2* expression levels. Both IGF-1R and Brg1 are enriched at the *SNAI2* promoter.

These studies further expand our knowledge about IGF-1R in cancer. Figure 4 summarizes the findings in this thesis. To fully appreciate the notion that IGF-1R and RTKs in general exists in the nucleus, further studies describing the roles of nuclear IGF-1R and its

transportation is needed. Increased knowledge of nuclear IGF-1R would enable the development of novel strategies for cancer therapies.

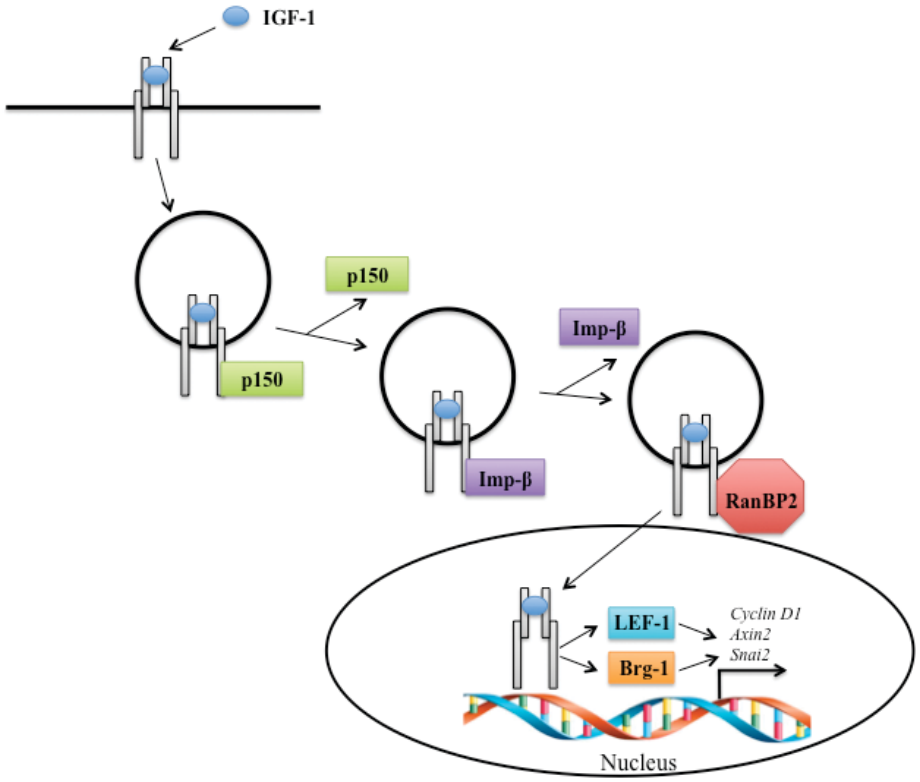

Figure 4: Schematic overview of nuclear transportation of IGF-1R and its nuclear functions. IGF-1 binds and activates IGF-1R at the plasma membrane. Activated IGF-1R is endocytosed and transported along microtubules with help of the retrograde transport protein $p150^{Glued}$. Close to the nuclear periphery IGF-1R is recognized and bound by importin-β. Importin-β brings IGF-1R to RanBP2. RanBP2 SUMOylates IGF-1R, thereby stabilizing it and enables IGF-1R to enter the nucleus through the NPC. In the nucleus IGF-1R induces expression of *cyclin D1, AXIN2* and *SNAI2* in collaboration with LEF-1 transcription factor and Brg1 chromatin remodeling protein.

5 ACKNOWLEDGEMENTS

It feels kind of strange that my period as a PhD student is coming to an end. The past couple of years flew by really fast. During the years I've been working at Karolinska Institutet I've had the opportunity to meet so many great people and I would like to thank everyone that has supported and helped me in any way or the other. I would especially like to thank:

My supervisor, **Olle Larsson**, for accepting me as a PhD student and introduce me to the interesting world of IGF-1R. By doing so you gave me the opportunity to do the thing I enjoy. I would also like to thank you for your laid back way of doing research and to allow me to work independently. I was able to take my projects in the direction I wanted and this made me enjoy research and science much more.

Bita, my co-supervisor and a great source of inspiration. I am very happy to know you and to have worked with you. You gave me so much valuable help and support during these years and I've learnt so much from you. Even though you left the lab after my first year as a PhD student, your knowledge, work ethics, energy and encouragement kept me going forward and helped me become the independent PhD student I am now. Thank you!

Yingbo, my co-supervisor. Thank you for always helping me whenever I needed help. Whether it was helping me changing cell medium when I was busy, fixing my computer or refresh my memory on how to do a certain lab technique, you were always available to help. I admire your calmness and positive energy, which is nice to have around.

Sylvia, my lab mate, I am very happy that I had a lab mate like you. You have always been very kind and helpful to me, which I am very thankful for. Especially during the period when I was extremely stressed and was "living" in the lab. Your knowledge and skills are impressive and since you are such a great team player, working with you was a pleasure. Bita was right when she said that she had a positive feeling about you before you joined the lab.

I would also like to thank past and present lab members: **Ahmed**, your working hours are weird! But that's also what's great and unique. **Sandra**, my co-supervisor when I was a master student and co-author on my first paper. Thank you for the nice and fun collaboration and helping me around during the early days. It was nice to have someone in the lab to talk football with. **Ann-Christin**, what a great student you are. You made my life as your supervisor so easy. Thank you for your contribution on my last project. Good luck with your studies! Also to **Sara L**, **Maria, Linda, Joana, Thomas, Eiman, Hua, Xiaoying, Zhiwei, Jennifer, Huiyuan, Daiana** and **Natalia.**

To past and present colleagues at CCK: **Jin**, **Marianne**, **Sofia**, **Hanif**, **Christos**, **Alex**, **Elisabeth**, **Sonyia**, **Salah**, **Anna**, **Nina**, **Cinzia**, **Emarn**, **Lidi**, **Qiang**, **Mei**, **Sophia**, **Fredrik**, **Susanne**, **Sofi**, **Diana**, **Nimesh**, **Shahab**, **Peng**, **Inga, Tong, Per**, **Masako**, **Mahdi**, **Sara H**, **Markus**, **Jeroen, Elin**, **Daniel**, **Naveen**, **Anna-Maria**, **Juan, Dali, Walid**, **Pádraig**, **Barry, Xiaonan**, **Roger**, **Deniz**, **Omid**, **Pinar**, **Nimrod** and **Hong.**

Ann-Britt Spåre for always being helpful with any kind of administrative issues. **Sören**, **Eva-Lena, Elle** and **Elisabeth**, without you CCK would probably not function so smoothly. Current and previous head of the Department of Oncology-Pathology, **Dan Grandér** and **Tina Dalianis** for providing a nice environment.

My previous supervisor and current mentor **Viljar Jaks**. You thought me lots of things when I was working at KI Southside.

Tina Persson and **Anethe Mansén** at KI Career Service and **Charlotte Bäckström (**previously at KI Career Service) at the KI Communications Office for the great ongoing collaboration with the Digital Ambassador program.

Matti Nikkola and **Carsten Daub** for organizing the excellent KI-RIKEN Yokohama joint course at the RIKEN Institute. **Erie Hayashi** for the nice collaboration during the course. We did a great job! And thanks for the matcha candy!

Elin Gunneriusson and **Susanne Klint** at Affibody for supervising me during my stay there.

Finally I would like to thank my **family** for all the support!

6 REFERENCES

1. Hanahan, D. and R.A. Weinberg, *The hallmarks of cancer.* Cell, 2000. **100**(1): p. 57-70.
2. Hanahan, D. and R.A. Weinberg, *Hallmarks of cancer: the next generation.* Cell, 2011. **144**(5): p. 646-74.
3. Deininger, P., *Genetic instability in cancer: caretaker and gatekeeper genes.* Ochsner J, 1999. **1**(4): p. 206-9.
4. Vogelstein, B. and K.W. Kinzler, *Cancer genes and the pathways they control.* Nat Med, 2004. **10**(8): p. 789-99.
5. Lengauer, C., K.W. Kinzler, and B. Vogelstein, *Genetic instabilities in human cancers.* Nature, 1998. **396**(6712): p. 643-9.
6. Smith, L., et al., *Duplication of ATR inhibits MyoD, induces aneuploidy and eliminates radiation-induced G1 arrest.* Nat Genet, 1998. **19**(1): p. 39-46.
7. Zhou, B.B. and S.J. Elledge, *The DNA damage response: putting checkpoints in perspective.* Nature, 2000. **408**(6811): p. 433-9.
8. Weaver, Z., et al., *Mammary tumors in mice conditionally mutant for Brca1 exhibit gross genomic instability and centrosome amplification yet display a recurring distribution of genomic imbalances that is similar to human breast cancer.* Oncogene, 2002. **21**(33): p. 5097-107.
9. Lemmon, M.A. and J. Schlessinger, *Cell signaling by receptor tyrosine kinases.* Cell, 2010. **141**(7): p. 1117-34.
10. Blume-Jensen, P. and T. Hunter, *Oncogenic kinase signalling.* Nature, 2001. **411**(6835): p. 355-65.
11. Yarden, Y. and A. Ullrich, *Growth factor receptor tyrosine kinases.* Annu Rev Biochem, 1988. **57**: p. 443-78.
12. Schlessinger, J., *Signal transduction by allosteric receptor oligomerization.* Trends Biochem Sci, 1988. **13**(11): p. 443-7.
13. Lemmon, M.A. and J. Schlessinger, *Regulation of signal transduction and signal diversity by receptor oligomerization.* Trends Biochem Sci, 1994. **19**(11): p. 459-63.
14. Huse, M. and J. Kuriyan, *The conformational plasticity of protein kinases.* Cell, 2002. **109**(3): p. 275-82.
15. Nolen, B., S. Taylor, and G. Ghosh, *Regulation of protein kinases; controlling activity through activation segment conformation.* Mol Cell, 2004. **15**(5): p. 661-75.
16. Niu, X.L., K.G. Peters, and C.D. Kontos, *Deletion of the carboxyl terminus of Tie2 enhances kinase activity, signaling, and function. Evidence for an autoinhibitory mechanism.* J Biol Chem, 2002. **277**(35): p. 31768-73.
17. Till, J.H., et al., *Crystal structure of the MuSK tyrosine kinase: insights into receptor autoregulation.* Structure, 2002. **10**(9): p. 1187-96.
18. Chen, H., et al., *A molecular brake in the kinase hinge region regulates the activity of receptor tyrosine kinases.* Mol Cell, 2007. **27**(5): p. 717-30.

19. Schlessinger, J. and M.A. Lemmon, *SH2 and PTB domains in tyrosine kinase signaling.* Sci STKE, 2003. **2003**(191): p. RE12.
20. Zwick, E., J. Bange, and A. Ullrich, *Receptor tyrosine kinase signalling as a target for cancer intervention strategies.* Endocr Relat Cancer, 2001. **8**(3): p. 161-73.
21. Miranda, C., et al., *Gain of function mutations of RTK conserved residues display differential effects on NTRK1 kinase activity.* Oncogene, 2002. **21**(54): p. 8334-9.
22. Snuderl, M., et al., *Mosaic amplification of multiple receptor tyrosine kinase genes in glioblastoma.* Cancer Cell, 2011. **20**(6): p. 810-7.
23. Liao, R.G., et al., *Inhibitor-sensitive FGFR2 and FGFR3 mutations in lung squamous cell carcinoma.* Cancer Res, 2013. **73**(16): p. 5195-205.
24. Borisov, N., et al., *Systems-level interactions between insulin-EGF networks amplify mitogenic signaling.* Mol Syst Biol, 2009. **5**: p. 256.
25. Morgillo, F., et al., *Heterodimerization of insulin-like growth factor receptor/epidermal growth factor receptor and induction of survivin expression counteract the antitumor action of erlotinib.* Cancer Res, 2006. **66**(20): p. 10100-11.
26. Saito, Y., et al., *Receptor heterodimerization: essential mechanism for platelet-derived growth factor-induced epidermal growth factor receptor transactivation.* Mol Cell Biol, 2001. **21**(19): p. 6387-94.
27. Fujita, M., et al., *Cross-talk between integrin alpha6beta4 and insulin-like growth factor-1 receptor (IGF1R) through direct alpha6beta4 binding to IGF1 and subsequent alpha6beta4-IGF1-IGF1R ternary complex formation in anchorage-independent conditions.* J Biol Chem, 2012. **287**(15): p. 12491-500.
28. Ju, L. and C. Zhou, *Association of integrin beta1 and c-MET in mediating EGFR TKI gefitinib resistance in non-small cell lung cancer.* Cancer Cell Int, 2013. **13**(1): p. 15.
29. van Biesen, T., et al., *Receptor-tyrosine-kinase- and G beta gamma-mediated MAP kinase activation by a common signalling pathway.* Nature, 1995. **376**(6543): p. 781-4.
30. Playford, M.P., et al., *Insulin-like growth factor 1 regulates the location, stability, and transcriptional activity of beta-catenin.* Proc Natl Acad Sci U S A, 2000. **97**(22): p. 12103-8.
31. Sinor-Anderson, A. and L. Lillien, *Akt1 interacts with epidermal growth factor receptors and hedgehog signaling to increase stem/transit amplifying cells in the embryonic mouse cortex.* Dev Neurobiol, 2011. **71**(9): p. 759-71.
32. Yamaguchi, H., et al., *Signaling cross-talk in the resistance to HER family receptor targeted therapy.* Oncogene, 2014. **33**(9): p. 1073-81.
33. Cadigan, K.M. and R. Nusse, *Wnt signaling: a common theme in animal development.* Genes Dev, 1997. **11**(24): p. 3286-305.
34. Clevers, H. and R. Nusse, *Wnt/beta-catenin signaling and disease.* Cell, 2012. **149**(6): p. 1192-205.

35. Reya, T., et al., *A role for Wnt signalling in self-renewal of haematopoietic stem cells.* Nature, 2003. **423**(6938): p. 409-14.
36. Wang, J. and A. Wynshaw-Boris, *The canonical Wnt pathway in early mammalian embryogenesis and stem cell maintenance/differentiation.* Curr Opin Genet Dev, 2004. **14**(5): p. 533-9.
37. Nusse, R. and H.E. Varmus, *Many tumors induced by the mouse mammary tumor virus contain a provirus integrated in the same region of the host genome.* Cell, 1982. **31**(1): p. 99-109.
38. Nusslein-Volhard, C. and E. Wieschaus, *Mutations affecting segment number and polarity in Drosophila.* Nature, 1980. **287**(5785): p. 795-801.
39. Rijsewijk, F., et al., *The Drosophila homolog of the mouse mammary oncogene int-1 is identical to the segment polarity gene wingless.* Cell, 1987. **50**(4): p. 649-57.
40. Takada, R., et al., *Monounsaturated fatty acid modification of Wnt protein: its role in Wnt secretion.* Dev Cell, 2006. **11**(6): p. 791-801.
41. Tanaka, K., Y. Kitagawa, and T. Kadowaki, *Drosophila segment polarity gene product porcupine stimulates the posttranslational N-glycosylation of wingless in the endoplasmic reticulum.* J Biol Chem, 2002. **277**(15): p. 12816-23.
42. Franch-Marro, X., et al., *In vivo role of lipid adducts on Wingless.* J Cell Sci, 2008. **121**(Pt 10): p. 1587-92.
43. Janda, C.Y., et al., *Structural basis of Wnt recognition by Frizzled.* Science, 2012. **337**(6090): p. 59-64.
44. Dann, C.E., et al., *Insights into Wnt binding and signalling from the structures of two Frizzled cysteine-rich domains.* Nature, 2001. **412**(6842): p. 86-90.
45. Pinson, K.I., et al., *An LDL-receptor-related protein mediates Wnt signalling in mice.* Nature, 2000. **407**(6803): p. 535-8.
46. Tamai, K., et al., *LDL-receptor-related proteins in Wnt signal transduction.* Nature, 2000. **407**(6803): p. 530-5.
47. Li, V.S., et al., *Wnt signaling through inhibition of beta-catenin degradation in an intact Axin1 complex.* Cell, 2012. **149**(6): p. 1245-56.
48. Behrens, J., et al., *Functional interaction of beta-catenin with the transcription factor LEF-1.* Nature, 1996. **382**(6592): p. 638-42.
49. Roose, J., et al., *The Xenopus Wnt effector XTcf-3 interacts with Groucho-related transcriptional repressors.* Nature, 1998. **395**(6702): p. 608-12.
50. Shtutman, M., et al., *The cyclin D1 gene is a target of the beta-catenin/LEF-1 pathway.* Proc Natl Acad Sci U S A, 1999. **96**(10): p. 5522-7.
51. Yan, D., et al., *Elevated expression of axin2 and hnkd mRNA provides evidence that Wnt/beta -catenin signaling is activated in human colon tumors.* Proc Natl Acad Sci U S A, 2001. **98**(26): p. 14973-8.
52. He, T.C., et al., *Identification of c-MYC as a target of the APC pathway.* Science, 1998. **281**(5382): p. 1509-12.

53. Peifer, M., et al., *The vertebrate adhesive junction proteins beta-catenin and plakoglobin and the Drosophila segment polarity gene armadillo form a multigene family with similar properties.* J Cell Biol, 1992. **118**(3): p. 681-91.
54. Tian, X., et al., *E-cadherin/beta-catenin complex and the epithelial barrier.* J Biomed Biotechnol, 2011. **2011**: p. 567305.
55. Krejci, P., et al., *Receptor tyrosine kinases activate canonical WNT/beta-catenin signaling via MAP kinase/LRP6 pathway and direct beta-catenin phosphorylation.* PLoS One, 2012. **7**(4): p. e35826.
56. Dingwall, C., S.V. Sharnick, and R.A. Laskey, *A polypeptide domain that specifies migration of nucleoplasmin into the nucleus.* Cell, 1982. **30**(2): p. 449-58.
57. Kalderon, D., et al., *A short amino acid sequence able to specify nuclear location.* Cell, 1984. **39**(3 Pt 2): p. 499-509.
58. Robbins, J., et al., *Two interdependent basic domains in nucleoplasmin nuclear targeting sequence: identification of a class of bipartite nuclear targeting sequence.* Cell, 1991. **64**(3): p. 615-23.
59. Huber, O., et al., *Nuclear localization of beta-catenin by interaction with transcription factor LEF-1.* Mech Dev, 1996. **59**(1): p. 3-10.
60. Molenaar, M., et al., *XTcf-3 transcription factor mediates beta-catenin-induced axis formation in Xenopus embryos.* Cell, 1996. **86**(3): p. 391-9.
61. Fagotto, F., U. Gluck, and B.M. Gumbiner, *Nuclear localization signal-independent and importin/karyopherin-independent nuclear import of beta-catenin.* Curr Biol, 1998. **8**(4): p. 181-90.
62. Sharma, M., et al., *Specific armadillo repeat sequences facilitate beta-catenin nuclear transport in live cells via direct binding to nucleoporins Nup62, Nup153, and RanBP2/Nup358.* J Biol Chem, 2012. **287**(2): p. 819-31.
63. Olins, A.L. and D.E. Olins, *Spheroid chromatin units (v bodies).* Science, 1974. **183**(4122): p. 330-2.
64. Oudet, P., M. Gross-Bellard, and P. Chambon, *Electron microscopic and biochemical evidence that chromatin structure is a repeating unit.* Cell, 1975. **4**(4): p. 281-300.
65. Luger, K., M.L. Dechassa, and D.J. Tremethick, *New insights into nucleosome and chromatin structure: an ordered state or a disordered affair?* Nat Rev Mol Cell Biol, 2012. **13**(7): p. 436-47.
66. Davey, C.A., et al., *Solvent mediated interactions in the structure of the nucleosome core particle at 1.9 a resolution.* J Mol Biol, 2002. **319**(5): p. 1097-113.
67. Luger, K., et al., *Crystal structure of the nucleosome core particle at 2.8 A resolution.* Nature, 1997. **389**(6648): p. 251-60.
68. Rohs, R., et al., *The role of DNA shape in protein-DNA recognition.* Nature, 2009. **461**(7268): p. 1248-53.
69. Zheng, C., et al., *Salt-dependent intra- and internucleosomal interactions of the H3 tail domain in a model oligonucleosomal array.* J Biol Chem, 2005. **280**(39): p. 33552-7.

70. Arents, G. and E.N. Moudrianakis, *The histone fold: a ubiquitous architectural motif utilized in DNA compaction and protein dimerization.* Proc Natl Acad Sci U S A, 1995. **92**(24): p. 11170-4.
71. Bannister, A.J. and T. Kouzarides, *Regulation of chromatin by histone modifications.* Cell Res, 2011. **21**(3): p. 381-95.
72. van Steensel, B., *Chromatin: constructing the big picture.* EMBO J, 2011. **30**(10): p. 1885-95.
73. Allfrey, V.G., R. Faulkner, and A.E. Mirsky, *Acetylation and Methylation of Histones and Their Possible Role in the Regulation of Rna Synthesis.* Proc Natl Acad Sci U S A, 1964. **51**: p. 786-94.
74. Dawson, M.A., et al., *JAK2 phosphorylates histone H3Y41 and excludes HP1alpha from chromatin.* Nature, 2009. **461**(7265): p. 819-22.
75. Tropberger, P. and R. Schneider, *Going global: novel histone modifications in the globular domain of H3.* Epigenetics, 2010. **5**(2): p. 112-7.
76. Xu, F., K. Zhang, and M. Grunstein, *Acetylation in histone H3 globular domain regulates gene expression in yeast.* Cell, 2005. **121**(3): p. 375-85.
77. Shi, Y., et al., *Histone demethylation mediated by the nuclear amine oxidase homolog LSD1.* Cell, 2004. **119**(7): p. 941-53.
78. Ng, S.S., et al., *Dynamic protein methylation in chromatin biology.* Cell Mol Life Sci, 2009. **66**(3): p. 407-22.
79. Clayton, A.L. and L.C. Mahadevan, *MAP kinase-mediated phosphoacetylation of histone H3 and inducible gene regulation.* FEBS Lett, 2003. **546**(1): p. 51-8.
80. Clayton, A.L., et al., *Phosphoacetylation of histone H3 on c-fos- and c-jun-associated nucleosomes upon gene activation.* EMBO J, 2000. **19**(14): p. 3714-26.
81. Walter, W., et al., *14-3-3 interaction with histone H3 involves a dual modification pattern of phosphoacetylation.* Mol Cell Biol, 2008. **28**(8): p. 2840-9.
82. Lee, J.S., et al., *Histone crosstalk between H2B monoubiquitination and H3 methylation mediated by COMPASS.* Cell, 2007. **131**(6): p. 1084-96.
83. Strahl, B.D. and C.D. Allis, *The language of covalent histone modifications.* Nature, 2000. **403**(6765): p. 41-5.
84. Kadam, S. and B.M. Emerson, *Transcriptional specificity of human SWI/SNF BRG1 and BRM chromatin remodeling complexes.* Mol Cell, 2003. **11**(2): p. 377-89.
85. Reyes, J.C., et al., *Altered control of cellular proliferation in the absence of mammalian brahma (SNF2alpha).* EMBO J, 1998. **17**(23): p. 6979-91.
86. Bultman, S., et al., *A Brg1 null mutation in the mouse reveals functional differences among mammalian SWI/SNF complexes.* Mol Cell, 2000. **6**(6): p. 1287-95.
87. de la Serna, I.L., K.A. Carlson, and A.N. Imbalzano, *Mammalian SWI/SNF complexes promote MyoD-mediated muscle differentiation.* Nat Genet, 2001. **27**(2): p. 187-90.
88. Willis, M.S., et al., *Functional redundancy of SWI/SNF catalytic subunits in maintaining vascular endothelial cells in the adult heart.* Circ Res, 2012. **111**(5): p. e111-22.

89. Federici, M., et al., *Distribution of insulin/insulin-like growth factor-I hybrid receptors in human tissues.* Mol Cell Endocrinol, 1997. **129**(2): p. 121-6.
90. LeRoith, D. and C.T. Roberts, Jr., *The insulin-like growth factor system and cancer.* Cancer Lett, 2003. **195**(2): p. 127-37.
91. Dufourny, B., et al., *Mitogenic signaling of insulin-like growth factor I in MCF-7 human breast cancer cells requires phosphatidylinositol 3-kinase and is independent of mitogen-activated protein kinase.* J Biol Chem, 1997. **272**(49): p. 31163-71.
92. Yang, Z., et al., *Genes involved in the immediate early response and epithelial-mesenchymal transition are regulated by adipocytokines in the female reproductive tract.* Mol Reprod Dev, 2012. **79**(2): p. 128-37.
93. Minshall, C., et al., *IL-4 and insulin-like growth factor-I inhibit the decline in Bcl-2 and promote the survival of IL-3-deprived myeloid progenitors.* J Immunol, 1997. **159**(3): p. 1225-32.
94. Hankinson, S.E., et al., *Circulating concentrations of insulin-like growth factor-I and risk of breast cancer.* Lancet, 1998. **351**(9113): p. 1393-6.
95. Ma, J., et al., *Prospective study of colorectal cancer risk in men and plasma levels of insulin-like growth factor (IGF)-I and IGF-binding protein-3.* J Natl Cancer Inst, 1999. **91**(7): p. 620-5.
96. Stattin, P., et al., *Plasma insulin-like growth factor-I, insulin-like growth factor-binding proteins, and prostate cancer risk: a prospective study.* J Natl Cancer Inst, 2000. **92**(23): p. 1910-7.
97. Sivakumar, R., et al., *Autocrine loop for IGF-I receptor signaling in SLUG-mediated epithelial-mesenchymal transition.* Int J Oncol, 2009. **34**(2): p. 329-38.
98. Vella, V., et al., *A novel autocrine loop involving IGF-II and the insulin receptor isoform-A stimulates growth of thyroid cancer.* J Clin Endocrinol Metab, 2002. **87**(1): p. 245-54.
99. Jansen, M., et al., *Sequence of cDNA encoding human insulin-like growth factor I precursor.* Nature, 1983. **306**(5943): p. 609-11.
100. Hoppener, J.W., et al., *The human gene encoding insulin-like growth factor I is located on chromosome 12.* Hum Genet, 1985. **69**(2): p. 157-60.
101. Rinderknecht, E. and R.E. Humbel, *The amino acid sequence of human insulin-like growth factor I and its structural homology with proinsulin.* J Biol Chem, 1978. **253**(8): p. 2769-76.
102. Tannenbaum, G.S. and N. Ling, *The interrelationship of growth hormone (GH)-releasing factor and somatostatin in generation of the ultradian rhythm of GH secretion.* Endocrinology, 1984. **115**(5): p. 1952-7.
103. Cohen, P., *Overview of the IGF-I system.* Horm Res, 2006. **65 Suppl 1**: p. 3-8.
104. Ekstrom, T.J., et al., *Promoter-specific IGF2 imprinting status and its plasticity during human liver development.* Development, 1995. **121**(2): p. 309-16.
105. Bjornsson, H.T., et al., *Epigenetic specificity of loss of imprinting of the IGF2 gene in Wilms tumors.* J Natl Cancer Inst, 2007. **99**(16): p. 1270-3.

106. Kaneda, A. and A.P. Feinberg, *Loss of imprinting of IGF2: a common epigenetic modifier of intestinal tumor risk.* Cancer Res, 2005. **65**(24): p. 11236-40.
107. Gluckman, P.D. and C.S. Pinal, *Regulation of fetal growth by the somatotrophic axis.* J Nutr, 2003. **133**(5 Suppl 2): p. 1741S-1746S.
108. Clemmons, D.R., *Involvement of insulin-like growth factor-I in the control of glucose homeostasis.* Curr Opin Pharmacol, 2006. **6**(6): p. 620-5.
109. Hwa, V., Y. Oh, and R.G. Rosenfeld, *The insulin-like growth factor-binding protein (IGFBP) superfamily.* Endocr Rev, 1999. **20**(6): p. 761-87.
110. Jones, J.I., et al., *Insulin-like growth factor binding protein 1 stimulates cell migration and binds to the alpha 5 beta 1 integrin by means of its Arg-Gly-Asp sequence.* Proc Natl Acad Sci U S A, 1993. **90**(22): p. 10553-7.
111. Leal, S.M., et al., *The type V transforming growth factor beta receptor is the putative insulin-like growth factor-binding protein 3 receptor.* J Biol Chem, 1997. **272**(33): p. 20572-6.
112. Rajah, R., et al., *Insulin-like growth factor binding protein (IGFBP) proteases: functional regulators of cell growth.* Prog Growth Factor Res, 1995. **6**(2-4): p. 273-84.
113. Cooke, R.M., T.S. Harvey, and I.D. Campbell, *Solution structure of human insulin-like growth factor 1: a nuclear magnetic resonance and restrained molecular dynamics study.* Biochemistry, 1991. **30**(22): p. 5484-91.
114. Abbott, A.M., et al., *Insulin-like growth factor I receptor gene structure.* J Biol Chem, 1992. **267**(15): p. 10759-63.
115. Sarfstein, R., et al., *Insulin-like growth factor-I receptor (IGF-IR) translocates to nucleus and autoregulates IGF-IR gene expression in breast cancer cells.* J Biol Chem, 2012. **287**(4): p. 2766-76.
116. Kato, H., et al., *Role of tyrosine kinase activity in signal transduction by the insulin-like growth factor-I (IGF-I) receptor. Characterization of kinase-deficient IGF-I receptors and the action of an IGF-I-mimetic antibody (alpha IR-3).* J Biol Chem, 1993. **268**(4): p. 2655-61.
117. Favelyukis, S., et al., *Structure and autoregulation of the insulin-like growth factor 1 receptor kinase.* Nat Struct Biol, 2001. **8**(12): p. 1058-63.
118. Dews, M., et al., *Domains of the insulin-like growth factor I receptor required for the activation of extracellular signal-regulated kinases.* Endocrinology, 2000. **141**(4): p. 1289-300.
119. Miura, M., S. Li, and R. Baserga, *Effect of a mutation at tyrosine 950 of the insulin-like growth factor I receptor on the growth and transformation of cells.* Cancer Res, 1995. **55**(3): p. 663-7.
120. Miura, M., et al., *Different effects on mitogenesis and transformation of a mutation at tyrosine 1251 of the insulin-like growth factor I receptor.* J Biol Chem, 1995. **270**(38): p. 22639-44.
121. O'Connor, R., et al., *Identification of domains of the insulin-like growth factor I receptor that are required for protection from apoptosis.* Mol Cell Biol, 1997. **17**(1): p. 427-35.

122. Leahy, M., et al., *Impaired Shc, Ras, and MAPK activation but normal Akt activation in FL5.12 cells expressing an insulin-like growth factor I receptor mutated at tyrosines 1250 and 1251.* J Biol Chem, 2004. **279**(18): p. 18306-13.
123. Kelly, G.M., et al., *Serine phosphorylation of the insulin-like growth factor I (IGF-1) receptor C-terminal tail restrains kinase activity and cell growth.* J Biol Chem, 2012. **287**(33): p. 28180-94.
124. Esposito, D.L., et al., *Tyr(612) and Tyr(632) in human insulin receptor substrate-1 are important for full activation of insulin-stimulated phosphatidylinositol 3-kinase activity and translocation of GLUT4 in adipose cells.* Endocrinology, 2001. **142**(7): p. 2833-40.
125. Alessi, D.R., et al., *Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase Balpha.* Curr Biol, 1997. **7**(4): p. 261-9.
126. Sarbassov, D.D., et al., *Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex.* Science, 2005. **307**(5712): p. 1098-101.
127. Feng, J., et al., *Identification of a PKB/Akt hydrophobic motif Ser-473 kinase as DNA-dependent protein kinase.* J Biol Chem, 2004. **279**(39): p. 41189-96.
128. Zhang, X., et al., *Akt, FoxO and regulation of apoptosis.* Biochim Biophys Acta, 2011. **1813**(11): p. 1978-86.
129. Nascimento, E.B., et al., *Phosphorylation of PRAS40 on Thr246 by PKB/AKT facilitates efficient phosphorylation of Ser183 by mTORC1.* Cell Signal, 2010. **22**(6): p. 961-7.
130. Vander Haar, E., et al., *Insulin signalling to mTOR mediated by the Akt/PKB substrate PRAS40.* Nat Cell Biol, 2007. **9**(3): p. 316-23.
131. Cushman, S.W. and L.J. Wardzala, *Potential mechanism of insulin action on glucose transport in the isolated rat adipose cell. Apparent translocation of intracellular transport systems to the plasma membrane.* J Biol Chem, 1980. **255**(10): p. 4758-62.
132. Andjelkovic, M., et al., *Activation and phosphorylation of a pleckstrin homology domain containing protein kinase (RAC-PK/PKB) promoted by serum and protein phosphatase inhibitors.* Proc Natl Acad Sci U S A, 1996. **93**(12): p. 5699-704.
133. Brognard, J., et al., *PHLPP and a second isoform, PHLPP2, differentially attenuate the amplitude of Akt signaling by regulating distinct Akt isoforms.* Mol Cell, 2007. **25**(6): p. 917-31.
134. Stambolic, V., et al., *Negative regulation of PKB/Akt-dependent cell survival by the tumor suppressor PTEN.* Cell, 1998. **95**(1): p. 29-39.
135. Qiao, L.Y., et al., *In vivo phosphorylation of insulin receptor substrate 1 at serine 789 by a novel serine kinase in insulin-resistant rodents.* J Biol Chem, 2002. **277**(29): p. 26530-9.
136. Greene, M.W., et al., *Modulation of insulin-stimulated degradation of human insulin receptor substrate-1 by Serine 312 phosphorylation.* J Biol Chem, 2003. **278**(10): p. 8199-211.

137. Terai, K. and M. Matsuda, *Ras binding opens c-Raf to expose the docking site for mitogen-activated protein kinase kinase.* EMBO Rep, 2005. **6**(3): p. 251-5.
138. Wu, X., et al., *Selective activation of MEK1 but not MEK2 by A-Raf from epidermal growth factor-stimulated Hela cells.* J Biol Chem, 1996. **271**(6): p. 3265-71.
139. Marais, R., et al., *Differential regulation of Raf-1, A-Raf, and B-Raf by oncogenic ras and tyrosine kinases.* J Biol Chem, 1997. **272**(7): p. 4378-83.
140. Payne, D.M., et al., *Identification of the regulatory phosphorylation sites in pp42/mitogen-activated protein kinase (MAP kinase).* EMBO J, 1991. **10**(4): p. 885-92.
141. Coles, L.C. and P.E. Shaw, *PAK1 primes MEK1 for phosphorylation by Raf-1 kinase during cross-cascade activation of the ERK pathway.* Oncogene, 2002. **21**(14): p. 2236-44.
142. Rossomando, A.J., et al., *Mitogen-activated protein kinase kinase 1 (MKK1) is negatively regulated by threonine phosphorylation.* Mol Cell Biol, 1994. **14**(3): p. 1594-602.
143. Sontag, E., et al., *The interaction of SV40 small tumor antigen with protein phosphatase 2A stimulates the map kinase pathway and induces cell proliferation.* Cell, 1993. **75**(5): p. 887-97.
144. Alessi, D.R., et al., *Inactivation of p42 MAP kinase by protein phosphatase 2A and a protein tyrosine phosphatase, but not CL100, in various cell lines.* Curr Biol, 1995. **5**(3): p. 283-95.
145. Pulido, R., A. Zuniga, and A. Ullrich, *PTP-SL and STEP protein tyrosine phosphatases regulate the activation of the extracellular signal-regulated kinases ERK1 and ERK2 by association through a kinase interaction motif.* EMBO J, 1998. **17**(24): p. 7337-50.
146. Sun, H., et al., *MKP-1 (3CH134), an immediate early gene product, is a dual specificity phosphatase that dephosphorylates MAP kinase in vivo.* Cell, 1993. **75**(3): p. 487-93.
147. Werner, H., et al., *Developmental regulation of the rat insulin-like growth factor I receptor gene.* Proc Natl Acad Sci U S A, 1989. **86**(19): p. 7451-5.
148. Liu, J.P., et al., *Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf-1) and type 1 IGF receptor (Igf1r).* Cell, 1993. **75**(1): p. 59-72.
149. Sell, C., et al., *Simian virus 40 large tumor antigen is unable to transform mouse embryonic fibroblasts lacking type 1 insulin-like growth factor receptor.* Proc Natl Acad Sci U S A, 1993. **90**(23): p. 11217-21.
150. Lee, A.V. and D. Yee, *Targeting IGF-1R: at a crossroad.* Oncology (Williston Park), 2011. **25**(6): p. 535-6; discussion 551.
151. Chen, H.X. and E. Sharon, *IGF-1R as an anti-cancer target--trials and tribulations.* Chin J Cancer, 2013. **32**(5): p. 242-52.
152. Mahajan, R., et al., *A small ubiquitin-related polypeptide involved in targeting RanGAP1 to nuclear pore complex protein RanBP2.* Cell, 1997. **88**(1): p. 97-107.

153. Matunis, M.J., E. Coutavas, and G. Blobel, *A novel ubiquitin-like modification modulates the partitioning of the Ran-GTPase-activating protein RanGAP1 between the cytosol and the nuclear pore complex.* J Cell Biol, 1996. **135**(6 Pt 1): p. 1457-70.
154. Klenk, C., et al., *SUMO-1 controls the protein stability and the biological function of phosducin.* J Biol Chem, 2006. **281**(13): p. 8357-64.
155. Widagdo, J., et al., *SUMOylation of GTF2IRD1 regulates protein partner interactions and ubiquitin-mediated degradation.* PLoS One, 2012. **7**(11): p. e49283.
156. Besnault-Mascard, L., et al., *Caspase-8 sumoylation is associated with nuclear localization.* Oncogene, 2005. **24**(20): p. 3268-73.
157. van der Veen, A.G. and H.L. Ploegh, *Ubiquitin-like proteins.* Annu Rev Biochem, 2012. **81**: p. 323-57.
158. Saitoh, H. and J. Hinchey, *Functional heterogeneity of small ubiquitin-related protein modifiers SUMO-1 versus SUMO-2/3.* J Biol Chem, 2000. **275**(9): p. 6252-8.
159. Tanaka, K., et al., *Characterization of a fission yeast SUMO-1 homologue, pmt3p, required for multiple nuclear events, including the control of telomere length and chromosome segregation.* Mol Cell Biol, 1999. **19**(12): p. 8660-72.
160. Wong, K.H., et al., *Sumoylation in Aspergillus nidulans: sumO inactivation, overexpression and live-cell imaging.* Fungal Genet Biol, 2008. **45**(5): p. 728-37.
161. Alkuraya, F.S., et al., *SUMO1 haploinsufficiency leads to cleft lip and palate.* Science, 2006. **313**(5794): p. 1751.
162. Kim, J.H., et al., *Roles of sumoylation of a reptin chromatin-remodelling complex in cancer metastasis.* Nat Cell Biol, 2006. **8**(6): p. 631-9.
163. Kim, J.H., et al., *SUMOylation of pontin chromatin-remodeling complex reveals a signal integration code in prostate cancer cells.* Proc Natl Acad Sci U S A, 2007. **104**(52): p. 20793-8.
164. Alshareeda, A.T., et al., *SUMOylation proteins in breast cancer.* Breast Cancer Res Treat, 2014. **144**(3): p. 519-30.
165. Bernier-Villamor, V., et al., *Structural basis for E2-mediated SUMO conjugation revealed by a complex between ubiquitin-conjugating enzyme Ubc9 and RanGAP1.* Cell, 2002. **108**(3): p. 345-56.
166. Hickey, C.M., N.R. Wilson, and M. Hochstrasser, *Function and regulation of SUMO proteases.* Nat Rev Mol Cell Biol, 2012. **13**(12): p. 755-66.
167. Schulz, S., et al., *Ubiquitin-specific protease-like 1 (USPL1) is a SUMO isopeptidase with essential, non-catalytic functions.* EMBO Rep, 2012. **13**(10): p. 930-8.
168. Shin, E.J., et al., *DeSUMOylating isopeptidase: a second class of SUMO protease.* EMBO Rep, 2012. **13**(4): p. 339-46.
169. Huang, J., et al., *SUMO1 modification of PTEN regulates tumorigenesis by controlling its association with the plasma membrane.* Nat Commun, 2012. **3**: p. 911.
170. Tatham, M.H., et al., *Comparative proteomic analysis identifies a role for SUMO in protein quality control.* Sci Signal, 2011. **4**(178): p. rs4.

171. Kang, J.S., et al., *The type I TGF-beta receptor is covalently modified and regulated by sumoylation.* Nat Cell Biol, 2008. **10**(6): p. 654-64.
172. Sehat, B., et al., *SUMOylation mediates the nuclear translocation and signaling of the IGF-1 receptor.* Sci Signal, 2010. **3**(108): p. ra10.
173. Sundvall, M., et al., *Protein inhibitor of activated STAT3 (PIAS3) protein promotes SUMOylation and nuclear sequestration of the intracellular domain of ErbB4 protein.* J Biol Chem, 2012. **287**(27): p. 23216-26.
174. Rakowicz-Szulczynska, E.M., et al., *Chromatin binding of epidermal growth factor, nerve growth factor, and platelet-derived growth factor in cells bearing the appropriate surface receptors.* Proc Natl Acad Sci U S A, 1986. **83**(11): p. 3728-32.
175. Ni, C.Y., et al., *gamma -Secretase cleavage and nuclear localization of ErbB-4 receptor tyrosine kinase.* Science, 2001. **294**(5549): p. 2179-81.
176. Offterdinger, M., et al., *c-erbB-3: a nuclear protein in mammary epithelial cells.* J Cell Biol, 2002. **157**(6): p. 929-39.
177. Wang, S.C., et al., *Binding at and transactivation of the COX-2 promoter by nuclear tyrosine kinase receptor ErbB-2.* Cancer Cell, 2004. **6**(3): p. 251-61.
178. Rakowicz-Szulczynska, E.M., M. Herlyn, and H. Koprowski, *Nerve growth factor receptors in chromatin of melanoma cells, proliferating melanocytes, and colorectal carcinoma cells in vitro.* Cancer Res, 1988. **48**(24 Pt 1): p. 7200-6.
179. Johnston, C.L., et al., *Fibroblast growth factor receptors (FGFRs) localize in different cellular compartments. A splice variant of FGFR-3 localizes to the nucleus.* J Biol Chem, 1995. **270**(51): p. 30643-50.
180. Stachowiak, M.K., et al., *Nuclear localization of functional FGF receptor 1 in human astrocytes suggests a novel mechanism for growth factor action.* Brain Res Mol Brain Res, 1996. **38**(1): p. 161-5.
181. Podlecki, D.A., et al., *Nuclear translocation of the insulin receptor. A possible mediator of insulin's long term effects.* J Biol Chem, 1987. **262**(7): p. 3362-8.
182. Aleksic, T., et al., *Type 1 insulin-like growth factor receptor translocates to the nucleus of human tumor cells.* Cancer Res, 2010. **70**(16): p. 6412-9.
183. Chen, C.W. and D. Roy, *Up-regulation of nuclear IGF-I receptor by short term exposure of stilbene estrogen, diethylstilbestrol.* Mol Cell Endocrinol, 1996. **118**(1-2): p. 1-8.
184. Domingues, I., et al., *VEGFR2 translocates to the nucleus to regulate its own transcription.* PLoS One, 2011. **6**(9): p. e25668.
185. Matteucci, E., P. Bendinelli, and M.A. Desiderio, *Nuclear localization of active HGF receptor Met in aggressive MDA-MB231 breast carcinoma cells.* Carcinogenesis, 2009. **30**(6): p. 937-45.
186. Lin, S.Y., et al., *Nuclear localization of EGF receptor and its potential new role as a transcription factor.* Nat Cell Biol, 2001. **3**(9): p. 802-8.
187. Huo, L., et al., *RNA helicase A is a DNA-binding partner for EGFR-mediated transcriptional activation in the nucleus.* Proc Natl Acad Sci U S A, 2010. **107**(37): p. 16125-30.

188. Hanada, N., et al., *Co-regulation of B-Myb expression by E2F1 and EGF receptor.* Mol Carcinog, 2006. **45**(1): p. 10-7.
189. Hung, L.Y., et al., *Nuclear epidermal growth factor receptor (EGFR) interacts with signal transducer and activator of transcription 5 (STAT5) in activating Aurora-A gene expression.* Nucleic Acids Res, 2008. **36**(13): p. 4337-51.
190. Lo, H.W., et al., *Nuclear interaction of EGFR and STAT3 in the activation of the iNOS/NO pathway.* Cancer Cell, 2005. **7**(6): p. 575-89.
191. Wang, S.C., et al., *Tyrosine phosphorylation controls PCNA function through protein stability.* Nat Cell Biol, 2006. **8**(12): p. 1359-68.
192. Sundvall, M., et al., *Differential nuclear localization and kinase activity of alternative ErbB4 intracellular domains.* Oncogene, 2007. **26**(48): p. 6905-14.
193. Stachowiak, E.K., et al., *Nuclear accumulation of fibroblast growth factor receptors in human glial cells--association with cell proliferation.* Oncogene, 1997. **14**(18): p. 2201-11.
194. Chioni, A.M. and R. Grose, *FGFR1 cleavage and nuclear translocation regulates breast cancer cell behavior.* J Cell Biol, 2012. **197**(6): p. 801-17.
195. Fang, X., et al., *Control of CREB-binding protein signaling by nuclear fibroblast growth factor receptor-1: a novel mechanism of gene regulation.* J Biol Chem, 2005. **280**(31): p. 28451-62.
196. Giri, D.K., et al., *Endosomal transport of ErbB-2: mechanism for nuclear entry of the cell surface receptor.* Mol Cell Biol, 2005. **25**(24): p. 11005-18.
197. Lo, H.W., et al., *Nuclear-cytoplasmic transport of EGFR involves receptor endocytosis, importin beta1 and CRM1.* J Cell Biochem, 2006. **98**(6): p. 1570-83.
198. Liao, H.J. and G. Carpenter, *Role of the Sec61 translocon in EGF receptor trafficking to the nucleus and gene expression.* Mol Biol Cell, 2007. **18**(3): p. 1064-72.
199. Wang, Y.N., et al., *Membrane-bound trafficking regulates nuclear transport of integral epidermal growth factor receptor (EGFR) and ErbB-2.* J Biol Chem, 2012. **287**(20): p. 16869-79.
200. Hsu, S.C. and M.C. Hung, *Characterization of a novel tripartite nuclear localization sequence in the EGFR family.* J Biol Chem, 2007. **282**(14): p. 10432-40.
201. Myers, J.M., et al., *Nuclear trafficking of FGFR1: a role for the transmembrane domain.* J Cell Biochem, 2003. **88**(6): p. 1273-91.
202. Reilly, J.F. and P.A. Maher, *Importin beta-mediated nuclear import of fibroblast growth factor receptor: role in cell proliferation.* J Cell Biol, 2001. **152**(6): p. 1307-12.
203. Quarto, N., F.P. Finger, and D.B. Rifkin, *The NH2-terminal extension of high molecular weight bFGF is a nuclear targeting signal.* J Cell Physiol, 1991. **147**(2): p. 311-8.
204. Deng, H., et al., *Over-accumulation of nuclear IGF-1 receptor in tumor cells requires elevated expression of the receptor and the SUMO-conjugating enzyme Ubc9.* Biochem Biophys Res Commun, 2011. **404**(2): p. 667-71.

205. Wu, Y.C., M. Zhu, and D.M. Robertson, *Novel nuclear localization and potential function of insulin-like growth factor-1 receptor/insulin receptor hybrid in corneal epithelial cells.* PLoS One, 2012. **7**(8): p. e42483.
206. Hoa, N., et al., *Nuclear targeting of IGF-1 receptor in orbital fibroblasts from Graves' disease: apparent role of ADAM17.* PLoS One, 2012. **7**(4): p. e34173.
207. Chen, J., et al., *Functional significance of type 1 insulin-like growth factor-mediated nuclear translocation of the insulin receptor substrate-1 and beta-catenin.* J Biol Chem, 2005. **280**(33): p. 29912-20.
208. Schroer, T.A., *Dynactin.* Annu Rev Cell Dev Biol, 2004. **20**: p. 759-79.
209. Xiao, A., et al., *WSTF regulates the H2A.X DNA damage response via a novel tyrosine kinase activity.* Nature, 2009. **457**(7225): p. 57-62.
210. Cook, P.J., et al., *Tyrosine dephosphorylation of H2AX modulates apoptosis and survival decisions.* Nature, 2009. **458**(7238): p. 591-6.
211. Krishnan, N., et al., *Dephosphorylation of the C-terminal tyrosyl residue of the DNA damage-related histone H2A.X is mediated by the protein phosphatase eyes absent.* J Biol Chem, 2009. **284**(24): p. 16066-70.
212. Singh, R.K., et al., *Histone levels are regulated by phosphorylation and ubiquitylation-dependent proteolysis.* Nat Cell Biol, 2009. **11**(8): p. 925-33.
213. Lavigne, M., et al., *Interaction of HP1 and Brg1/Brm with the Globular Domain of Histone H3 Is Required for HP1-Mediated Repression.* Plos Gen, 2009. **5**(12): p 1-11.