1 INTRODUCTION

Cancer, regarded as “a wound that never heals”, is characterized by unchecked proliferation of abnormal cells and anomalous recognition of the immune system (Yin et al., 2021). It is a complex genetic disorder involving dynamic alterations in the genome (Grizzi and Chiriva-Internati, 2006). It is one of the leading cause of deaths globally, accounting for almost 10 million deaths in 2020 (Ferlay et al., 2021). One of the fundamental features of cancer is tumor formation. On the basis of histopathologic appearance and clinical behavior, tumors can be categorized as malignant or benign. Malignant tumors exhibit a poor level of tissue differentiation, resembling the primitive tissue they are derived from. Benign tumors on the other hand display good differentiation (Liu, 2018). Only malignant tumors are considered and properly referred to as cancers, and it is their ability to invade and metastasize that makes cancer a menace and so dangerous (Cooper, 2000). Tumors can also be categorized into embryonic or germ cell origin and an adult-organ origin.

Embryonal tumors and Neuroblastoma:

French biologists, Lobstein and Recamier, in 1882 theorized the concept of the embryonic origin of tumors for the first time. It was in 1970s, when Dr. Pierce proposed the theory ‘cancer, a developmental biology’ and pointed out that tumorigenesis concerned intimately with developmental biology to a great degree (Ma et al., 2010; Pierce, 1983). There are many different types of embryonic tumors, and it may include medulloblastoma, medulloepitheliomas, embryonal tumors with multilayered rosettes (ETMRs), Wilms tumor and even neuroblastoma. Neuroblastoma (NB) is a malignancy of the sympathetic nervous system that almost exclusively occurs in early childhood (Johnsen, Dyberg and Wickström, 2019). The cell of origin for

neuroblastoma has yet to be precisely defined but it probably derives from sympathoadrenal progenitor cells within the neural crest that differentiates to sympathetic ganglion cells and adrenal catecholamine-secreting chromaffin cells (Fig: 1.1) (L'Abbate et al., 2014). During embryogenesis, neural crest cells undergo epithelial-to-mesenchymal transition (EMT) enabling them to delaminate, migrate and differentiate into a broad range of cell types that contributes to anatomical structures within the organism. These processes are regulated by a complex set of external signaling, activation of transcriptional programs and epigenetic events. Dysregulation of these processes can induce changes in cell specification, migration and differentiation giving rise to hyperneoplastic lesions that in due course can result in neuroblastoma (Johnsen, Dyberg and Wickström, 2019).

Neuroblastoma was described by James Wright in 1910, and was named so because these cells were associated with fibrils in arrangements similar to neuroblasts (Huang and Weiss, 2013). It is the primary cause of death from pediatric cancer for children between the ages of one and five years (Louis and Shohet, 2015). The overall incidence of neuroblastoma is 6–7 cases per million and while it accounts for 6% of all childhood malignancies, neuroblastoma accounts for 15% of childhood cancer mortality (Piskareva and Stallings, 2015; Krystal, Sokol and Bagatell, 2022). However, these numbers likely underestimate its true occurance, as neuroblastoma regresses in some infants who therefore may never present to medical attention. The median age is ∼19 months at the time of diagnosis. Diagnosis can also be done *in utero* by fetal magnetic resonance imaging, or, hardly ever in patients older than 19 years (London et al., 2005; Louis and Shohet, 2015). Almost 40% of the patients are younger than 1 year at the time of diagnosis whereas less than 5% are older than 10 years (Matthay et al., 2016; Johnsen et al., 2018; Lundberg, Treis and Johnsen, 2022). Clinically, neuroblastoma manifests as a primary tumor anywhere along with the sympathetic nervous system, with > 50% occurring in the adrenal medulla (Park, Eggert and Caron, 2010).

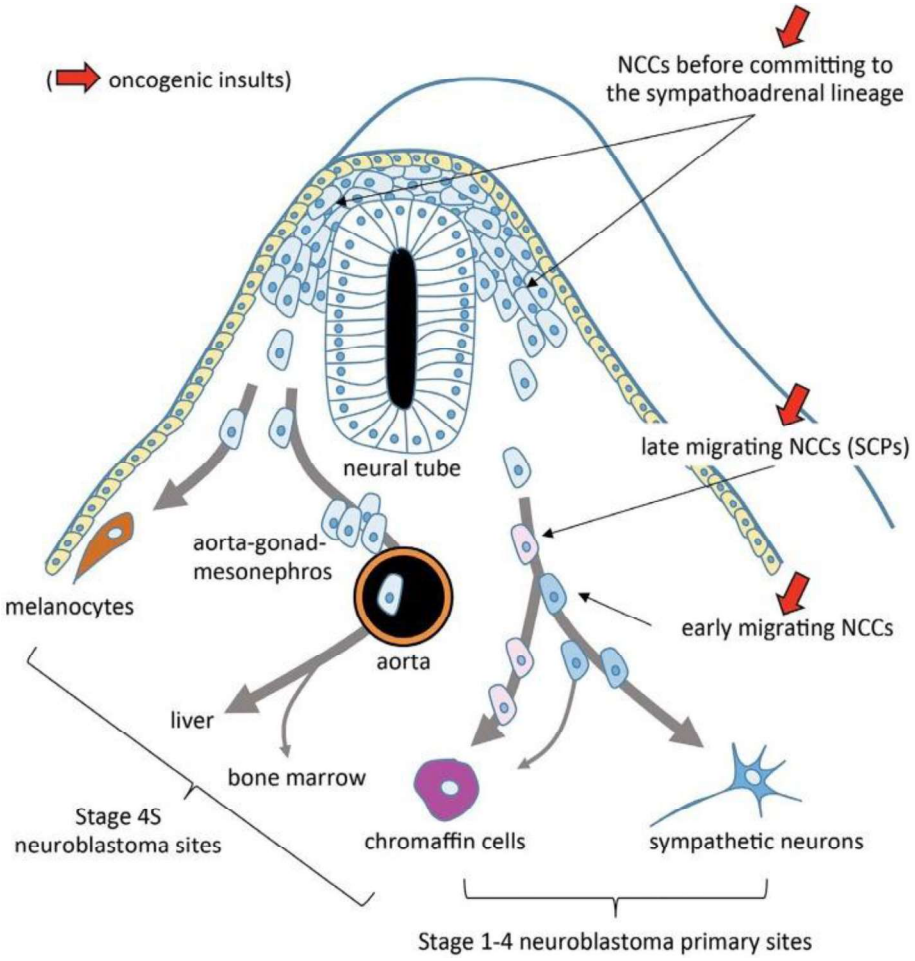

Fig. 1.1: Origins of Neuroblastoma

Image source: Tsubota & Kadomatsu, 2017

Neuroblastoma is distinguished from other solid tumors by its biologic heterogeneity and range of clinical behavior which spans from cases of highly aggressive metastatic disease unresponsive to the standard and investigational anticancer treatment to spontaneous regression. Because it is associated with contrasting patterns of clinical behavior, NB is often described as unpredictable and enigmatic (Mullassery et al., 2009). Although the biological and histological characteristics used for classification continue to evolve with new scientific data, they are used to divide patients into low-, intermediate-, and high-risk strata. Patients with low-risk disease, in general, have excellent overall survival rates with observation only or minimal therapeutic interventions. The

outcome of patients with intermediate-risk disease, who are mainly treated with surgery and chemotherapy, has improved to the point where many groups are focused on using biologic markers to help further decrease therapy in specific subpopulations of children (London et al., 2005; Park et al., 2013). And patients with high-risk disease comprise almost 50% of all new neuroblastoma cases each year (Lin et al., 2021).

Embryonic origins of Neuroblastoma:

Several experimental and clinical features link neuroblastoma to defective embryogenesis and neuroblast precancer cells. The baseline characteristics of NB at cellular and molecular level is Similar to that of the human fetal adrenal neuroblasts (de Preter et al., 2006; Marshall et al., 2014). Neuroblast progenitors migrate under the influence of MYCN and bone morphogenetic proteins (BMPs). They migrate from the neural crest and around the neural tube to a location that is immediately lateral to the notochord and dorsal aorta. The cells then undergo specification as the primary sympathetic ganglia (PSG) before divergence into neural cells of the mature sympathetic ganglia or chromaffin cells.

MYCN is a 'first hit' by virtue of the observations from a tyrosine hydroxylase (Th)–MYCN-transgenic mouse model, whereas mutations in anaplastic lymphoma kinase (ALK) and paired-like homeobox 2B (PHOX2B) are germline mutations (Calao et al., 2012). Local availability of nerve growth factor (NGF) determines whether a normal sympathetic ganglion cell will mature into a terminal ganglion or it will undergo apoptotic cell death. A relatively common pathological state is the postnatal survival of the precancer neuroblast cells. This requires the cell that is destined to become malignant to be resistant to trophic factor withdrawal before these persistent precancer cells undergo a third change to induce transformation. This transformation presents as NB in early childhood (Fig. 1.2) (Marshall et al., 2014).

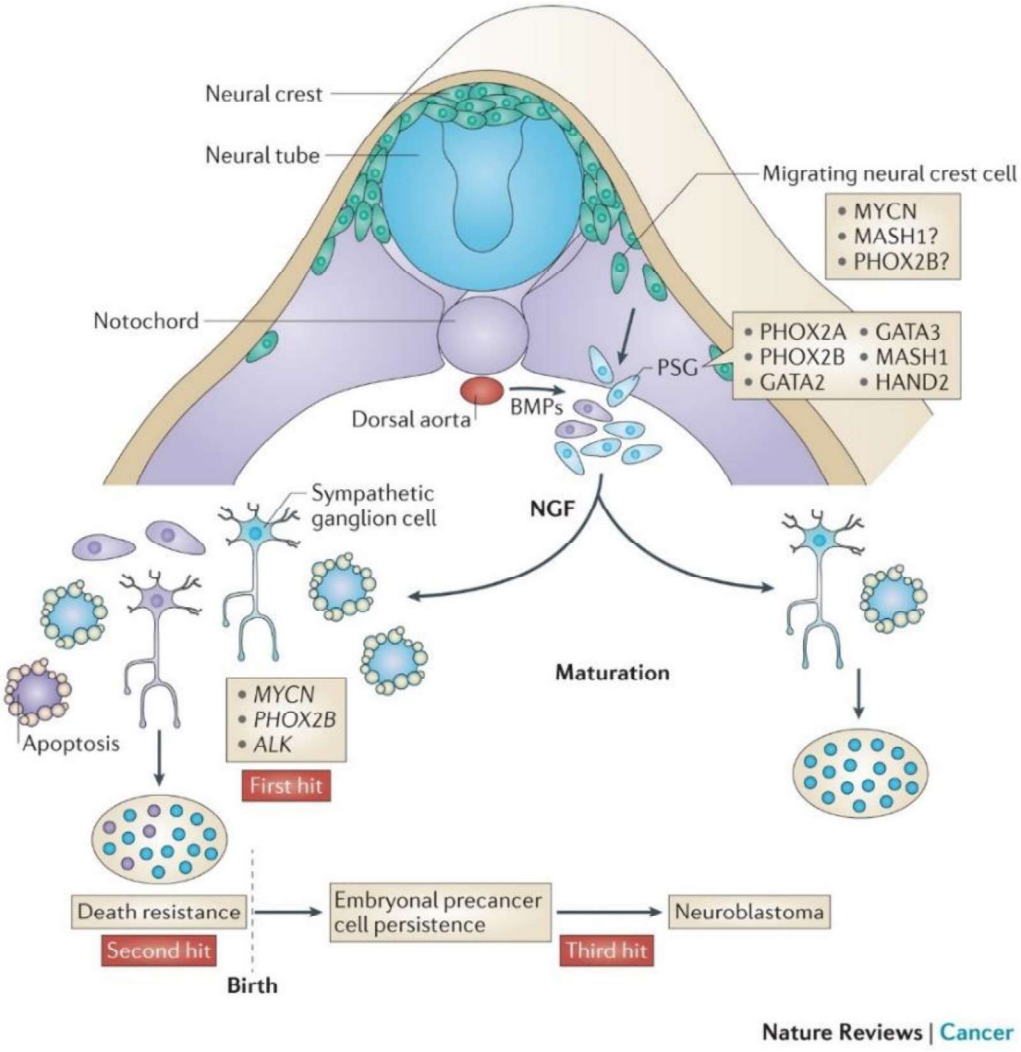

Fig. 1.2: Neural crest development and proposed Neuroblastoma development.

Image source: Marshall et al., 2014

High-risk Neuroblastoma (MYCN and ALK):

Established characteristics for high-risk neuroblastoma patients include age, loss of heterozygosity for chromosome 1p or 11q, unfavorable histopathology and amplification of MYCN (Fig. 1.3) (Mueller and Matthay, 2009; Huang and Weiss, 2013). Amplification of the MYCN oncogene (MNA) is the most robust genetic factor correlated with poor clinical outcome and can be found in about 16-20% of NB cases (and up to 40% in high-risk tumors) (Ambros et al., no date; Szewczyk et al., 2019).

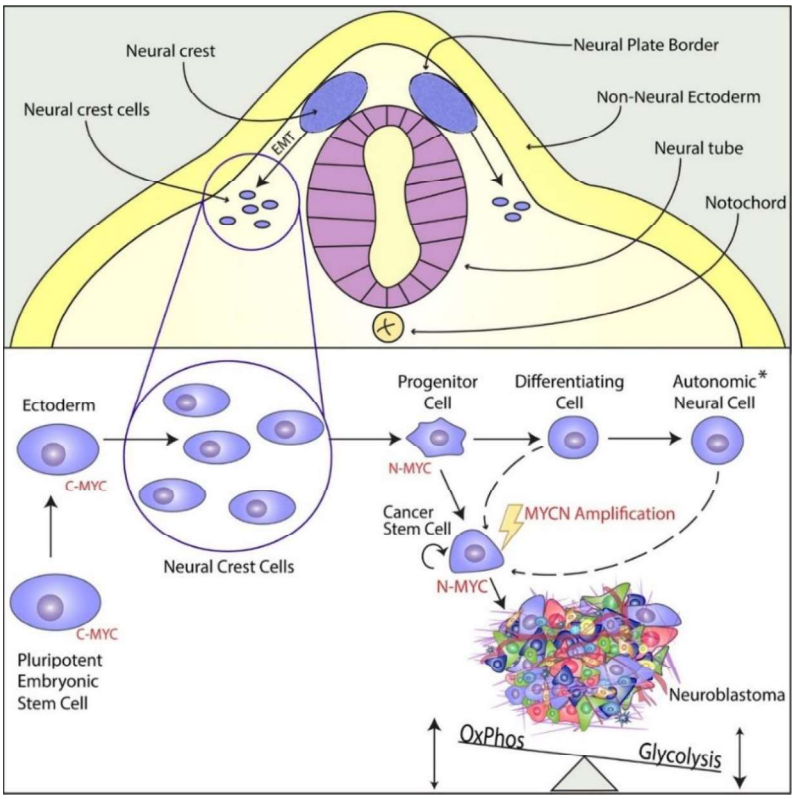

Fig. 1.3: N-MYC/MYCN acts as a Cancer Stem Cell Factor in the Developing Neural Crest and Promotes Tumorigenesis in Neuroblastoma

Image source: Otte et al., 2021

Activating mutations of the anaplastic lymphoma kinase (ALK) gene can also occur in NB, along with the amplification of the MYCN oncogene. The ALK gene resides on chromosome 2p23 centromeric to the MYCN locus on chromosome 2q24. Approximately 2-3% cases of NB have amplification of ALK and these occur almost invariably concomitant with amplification of MYCN (Azarova, Gautam and George, 2011; Kelleher, 2013). However, amplification of the *ALK* gene without concomitant *MYCN* gene amplification can occur, for example a particularly informative case of a neuroblastoma with a high-level amplicon involving and solely limited to the *ALK* gene was described by French investigators in 2008 in one of the landmark papers that established the importance of ALK in neuroblastoma (Janoueix-Lerosey et al., 2008; Kelleher, 2013). Using a transgenic zebrafish model of NB, a series of experiments on pathogenic cooperation between

ALK and MYCN were conducted in which MYCN induced tumor arose from a subpopulation of neuroblasts which migrates to the interrenal gland, the zebrafish equivalent of the adrenal medulla (Zhu et al., 2012). In this model co-expression of activated ALK with MYCN tripled the disease penetrance and markedly accelerated the tumor onset. Usually, MYCN overexpression blocks chromaffin cell differentiation, induces adrenal sympathetic neuroblast hyperplasia and ultimately triggers a developmentally-timed apoptotic response in the hyperplastic sympathoadrenal cells. However though, co-expression of activated ALK with MYCN provides pro-survival signals that block this apoptotic response and allow continued expansion and oncogenic transformation of hyperplastic neuroblasts, thus promoting progression to neuroblastoma (Zhu et al., 2012). Another study found that both wild-type and gain-of-function mutants in ALK are able to initiate mRNA transcription of the *MYCN* gene in both neuronal and neuroblastoma cell lines. It was also reported by this study that co-transfection of ALK gain-of-function mutations together with MYCN lead to an increase in transformation potential. This suggests that ALK signaling regulates initiation of transcription of the MYCN gene providing a possible explanation for the poor clinical outcome observed when MYCN is amplified together with activated ALK (Schönherr et al., 2012).

Growth factors and Neuroblastoma:

The development of an embryo requires intricate and precise control over cellular machinery and mechanisms to establish a structural and functional organism from the stem cells. Recent advances in stem cell research have disclosed the detailed processes of embryogenesis, stem cell differentiation, and cell reprogramming. In contrast to embryogenesis, cancer is considered a dysregulated cellular process. Particularly, cancer growth use many of the machinery originally used in the development processes, but they are deregulated for cancer growth and metastasis (Reya et al., 2001; Afify and Seno, 2019; Chen et al., 2021).

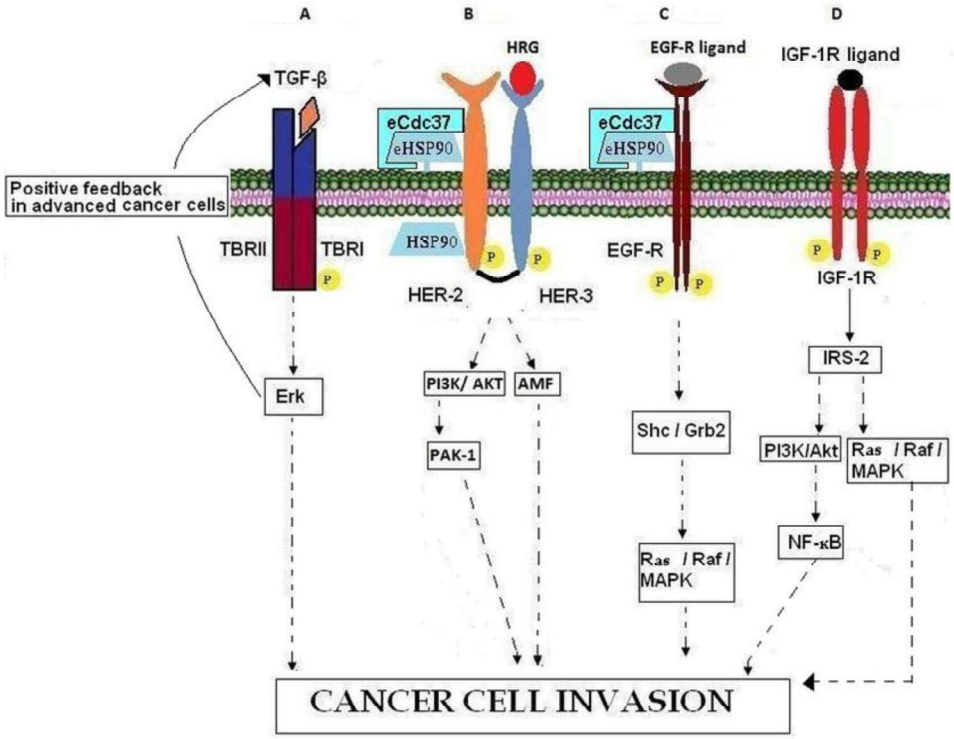

Fig. 1.4: Involvement of growth factors, growth factor receptors and chaperones in cancer cell invasion.

Image source: Stivarou & Patsavoudi, 2015

One common factor that is associated with embryonic development and cancer, are growth factors. Many pre-migratory neural crest cells are pluripotent and their potency for differentiation being gradually restricted as they migrate along definite pathways and interact with growth factors present in the microenvironment. Growth factors exert a direct influence on the differentiation of neural and other related neural crest-derived tissues (Hall and Ekanayake, 1991). Growth factors may be defined as any group of protein that stimulate the growth of specific tissues and play an important role in promoting cellular differentiation and cellular division (Shrivastava and Bhadauria, 2016). The eminent chronic proliferative property of cancer is imparted largely by growth factors. Growth factors maybe synthesized by cancer cells themselves or cancer cells may send signals to stimulate normal cells to secrete growth factors (Hanahan and Weinberg, 2011; Shrivastava and Bhadauria, 2016). Research suggests that growth factors can increase

transcription of certain proto-oncogene (myc and fos) (Sotiriou et al., 2003). Epidermal growth factor (EGF), transforming growth factor (TGF), platelet-derived growth factor (PDGF), hepatocyte growth factor (HGF), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), and insulin-like growth factor (IGF) stimulate cancer cell and stromal cell proliferation, migration, and invasion thereby promoting tumor growth, angiogenesis, and metastasis (Bach, 2018).

Insulin-like growth factor I Receptor signaling and Neuroblastomagenesis:

The components of the insulin-like growth factor (IGF) system, include IGFs (IGF-I and IGF-II), type I and type II IGF receptors, a family of six secreted IGF-binding proteins (IGFBPs) and IGFBP proteases (Jones and Clemmons, 1995; Agrogiannis et al., 2014). The six types of IGFBPs have been designated IGFBP-1 through IGFBP-6. Mammals, including humans generally possess one gene that belongs to each of the six types. All IGFBPs generally have approximately 200–300 amino acids and share a structure consisting of a highly cysteine-rich N-terminal domain that is highly evolutionary conserved among the IGFBP family and across species, a variable linker domain, and an evolutionary conserved cysteine-rich C-terminal domain (Allard and Duan, 2018). IGFBP modulates IGF action by functioning as IGF carriers within the circulation. Locally expressed IGFBPs also modulate IGF action by inhibiting binding to the IGF-IR (Forbes, McCarthy and Norton, 2012).

The IGFs are mitogenic single-chain polypeptides, structurally similar to proinsulin, that function as classical hormones and also in an autocrine/paracrine manner. The two IGF receptors are structurally and functionally unrelated. IGF ligand signaling is mediated by IGF-IR, which is a transmembrane glycoprotein with tyrosine kinase activity. IGF-IIR is a single-chain protein possessing no kinase activity (Agrogiannis et al., 2014). Upon ligand stimulation, IGF-IR undergoes autophosphorylation and phosphorylates adaptor proteins such as IRS1/2 and SHC; subsequently, it activates downstream PI3K/AKT, RAS/MAPK, and JAK/STAT signaling, which

modulate gene expressions in apoptosis as well as protein synthesis and cell proliferation (Chen et al., 2021; Girnita et al., 2014).

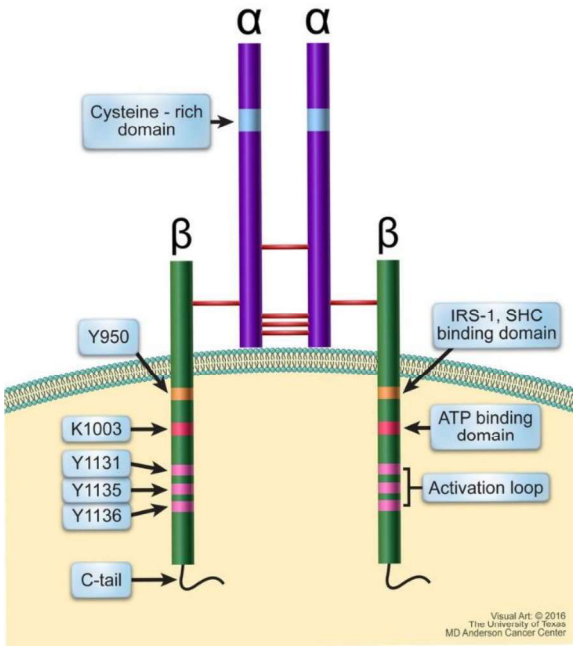

Fig. 1.5 Schematic diagram of IGF-IR

Image source: Vishwamitra et al., 2017

Activation of IGF signaling pathways promotes the growth, metastasis, and drug resistance in many types of human tumors, including mesenchymal, epithelial, and hematopoietic cancer (Hua et al., 2020). Physiologically, IGF-IR signaling is requisite during normal development. For example, IGF signaling plays a crucial role in regulating organ size. For postnatal growth, the growth hormone (GH)/IGF-I axis is essential, on a large scale. GH insufficiency and deficiency result in smaller body size during an individual's adulthood; furthermore, IGF-I expression in GH-deficient mice reverses the decreased body size caused by GH deficiency, suggesting that GH exerts its pro-growth function through IGF-I (Kaplan & Cohen, 2007; Velloso, 2008; Chen et al., 2021). The IGF-IR pathway has also been linked to cancer progression in multiple cancer types, including liver, lung, breast, colorectal and even neuroblastoma (van Golen et al., 2006 Dallas et al., 2009 Chang et al., 2013; Chang et al., 2015; Chen et al., 2021). Given the importance of IGF-

IR signaling as a core pathway in regulating multiple cellular responses, researchers are exploring the mechanism of how IGF-IR signaling is modulated.

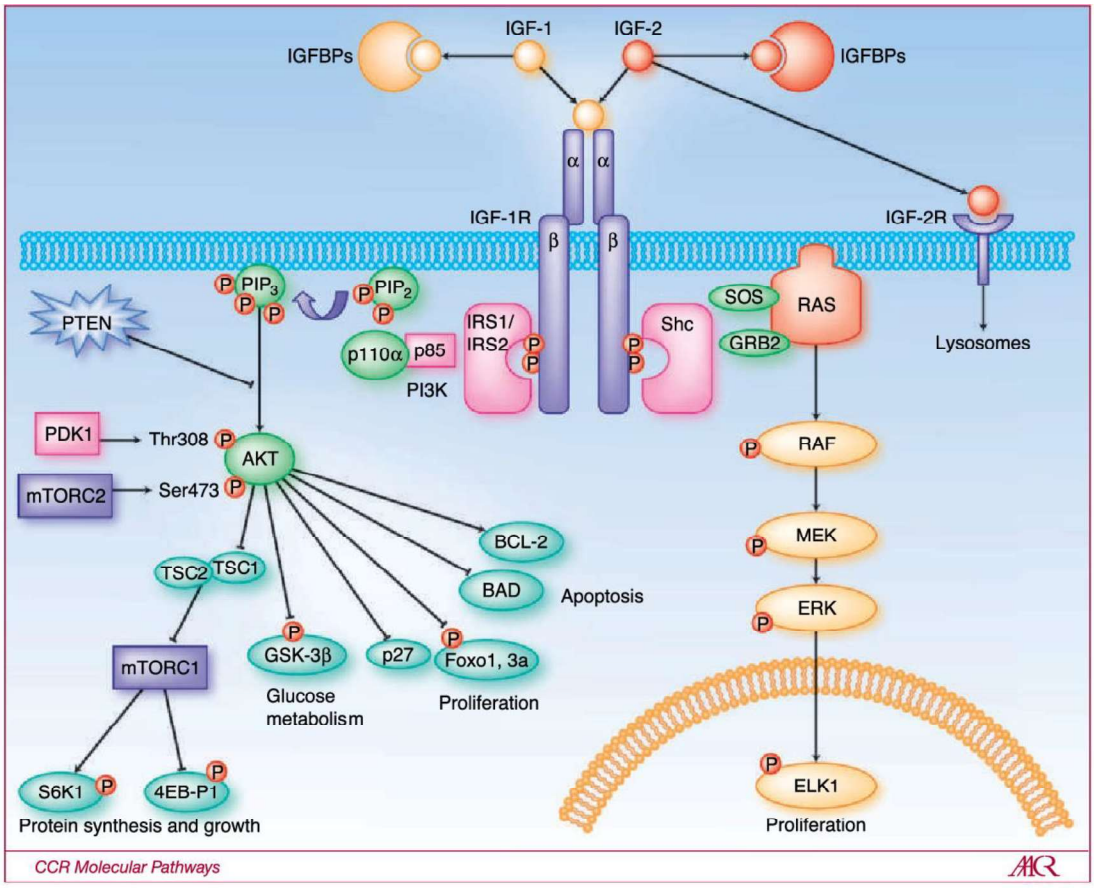

Fig. 1.6: Key components of the IGF-IR Pathway.

Image Source: Zha & Lackner, 2010

Key components of the IGF-IR pathway include both the ligands, IGF-I and IGF-II. Both of these ligands are capable of binding and stimulating the catalytic activity of the IGF-IR. Bioavailability of IGF-I is modulated by a family of IGFBPs, whereas bioavailability of IGF-II is modulated both by the IGFBPs and by binding to the IGF-IIR, which leads to receptor-mediated internalization and degradation of IGF-II in lysosomes. Upon binding by either IGF-I or IGF-II, the IGF-IR undergoes receptor cross-linking and autophosphorylation, leading to the creation of multiple

docking sites for the adaptor proteins IRS-I, IRS-II, and Shc. IRS-I and IRS-II binding results in activation of the class I phosphatidyl inositol 3′ kinase, whose catalytic activity is the conversion of PIP2 to the lipid second messenger PIP3. This event recruits the AKT family of kinases to the plasma membrane, where they can be phosphorylated and activated by PDK1 and the mTOR-containing complex mTORC2 (Neudauer et al., 2003, Menu et al., 2006). The activated AKT then mediates a host of cell signaling events, including disinhibition of the mTORC1 complex and increased protein synthesis and cell growth, increased conversion of glucose to glycogen via inhibition of GSK-3β, and increased proliferation and survival by activation or inhibition of key effectors such as the Foxo transcription factors, p27, BAD, and BCL-2 (Li et al., 2015). In contrast, Shc binding to activated IGF-1R results in stimulation of the RAS/MAP kinase pathway, which also leads to increased cell proliferation (Zha and Lackner, 2010).

IGF-IR signaling and Epithelial to Mesenchymal Transition:

Increasing amount of evidence indicates that the IGF-IR signaling is also involved in EMT-mediated tumor metastasis and drug resistance (Li et al., 2017). EMT is a multi-step biologic process characterized by the cell-cell contacts breakdown, cell-matrix adhesion remodeling and gain of mesenchymal phenotype (Thiery et al., 2009; Mitra, Mishra and Li, 2015; Li et al., 2017). Transformation from an epithelial cell into a mesenchymal cell needs alterations in cell morphology, cellular architecture, adhesion and migration ability. Loss of the epithelial marker E-cadherin and gain of mesenchymal marker vimentin are considered as the fundamental event in EMT processs (Nieto et al., 2016; Li et al., 2017). Down-regulation of E-cadherin expression causes adherens junctions breakdown between cells, loss of cell polarity, leading to a mesenchymal phenotype with invasive abilities (Wijnhoven, Dinjens and Pignatelli, 2000).

Many preclinical studies indicate that IGF-I contributes to metastasis in prostate, breast, lung and gastric cancer by inducing EMT. IGF-IR is involved in epidermal growth factor receptor (EGFR) TK inhibitor (TKI) resistance through crosstalk between IGF-IR and EMT signaling pathways in

non-small cell lung cancer (NSCLC) with EGFR mutations (Vazquez-Martin et al., 2013; Zhou et al., 2015; Li et al., 2017). In addition, IGF-IR signaling mediates resistance to TKI drugs targeting both epidermal growth factor receptor 2 (HER-2) and EGFR in gastric cancer via EMT-like process (Zhang et al., 2014; Li et al., 2017). Therefore, the close relationships between IGF-I/IGF-IR signaling and EMT progression makes it an alluring therapeutic target for cancer treatment.

The mechanism of IGF-IR signaling in regulation of EMT can be summed up in three aspects: autocrine ligand production and receptor overexpression, signal transduction by ligand binding, and cross-talk between signaling pathways (Li et al., 2017). Ligand activation of IGF-IR results in intrinsic tyrosine kinase phosphorylation which activates downstream adaptor protein IRS-1 and Shc, leading to activation of two main signaling pathways, Ras/Raf/ERK and IRS-1/PI3K/Akt pathways respectively (Laviola, Natalicchio and Giorgino, 2007; J. Ma et al., 2010a; Li et al., 2017). Activation of ERK pathway in response to IGF-I stimulation results in up-regulation of ZEB1 expression which induces EMT progression in prostate cancer (Dupont et al., 2001; Graham et al., 2008). Akt and ERK pathways are partially involved in IGF-I-induced EMT process in gastric cancer. Knockdown of Akt/ERK gene partially or inhibition of Akt/ERK pathways or reversed IGF-I-induced EMT through up-regulation of microRNA-200c which directly targets E-cadherin transcriptional repressors ZEB2 (Li et al., 2014). In addition to these two signaling pathways, GSK-3β is now considered as a crucial EMT regulator in response to IGF-I (Bachelder et al., 2005; Li et al., 2017).

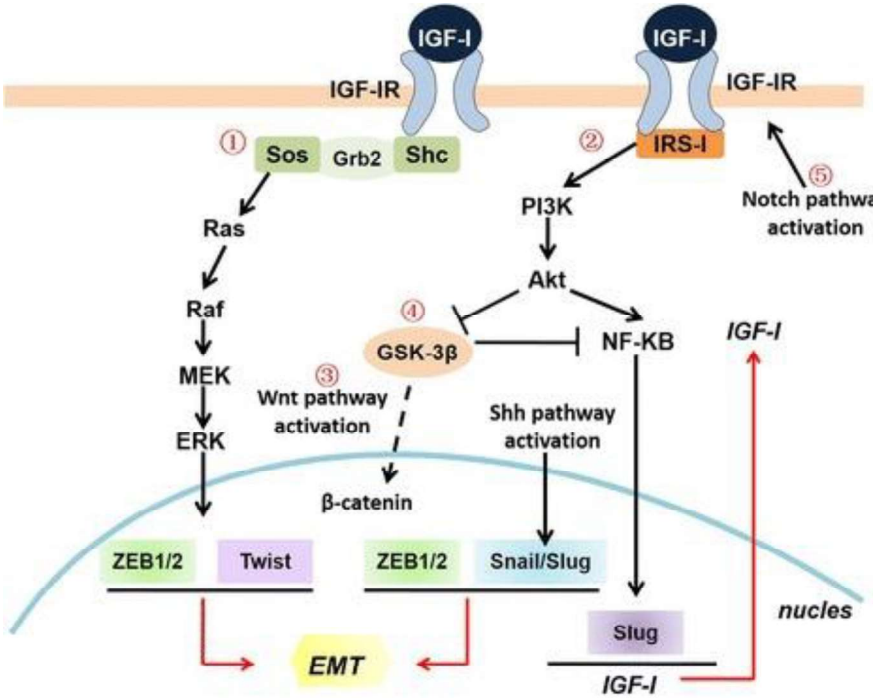

Fig. 1.7: Schematic representation of IGF signaling regulation in EMT

Image source: Li et al., 2017

Activation of ERK and Akt and pathways result in inactivation of GSK-3β in response to paracrine/autocrine IGF-I through Ser9 phosphorylation (Park, Kido and Accili, 1999; Ding et al., 2005). It was detected by Kem et al. that GSK-3β was involved in direct reduction of Snail and Slug expression through proteasome-dependent degradation or NF-κB activation in response to IGF-I stimulation (Kim et al., 2007; Li et al., 2017). Zhou et al. reported that GSK-3β could bind to and phosphorylate Snail at two consensus motifs to regulate the biological functions of Snail; activation of Akt pathway led to the suppression of GSK-3β through stabilization of Snail and phosphorylation of Ser9 in response of IGF-I (Zhou et al., 2004; Li et al., 2017). It has been shown that inhibition of Akt reversed IGF-I-induced EMT and mesenchymal phenotype in gastric cancer cells through initiating GSK-3β ability in epithelial phenotype maintenance (Li et al., 2015, 2017). These results indicate that the main signal transduction pathways by IGF-I ligand binding,

ERK/MAPK and IRS-1/Akt/GSK-3β pathways, are potent inducers/activators in IGF-I-induced EMT process. Fig. 1.7 represents the relationship between the IGF-I system and the EMT process.

Like other several cancers, IGF signaling was implicated in the survival of NB cells decades ago (El-Badry et al., 1989). In a study carried out by Wang et al., (2019), IGF-IR was shown to stimulate cancer stem cell-like features in NB cells via the regulation of the AKT and STAT3 pathways (Wang et al., 2019).

MicroRNAs and Cancer:

MicroRNAs (miRNAs) are small non-coding regulatory RNAs with sizes of 17-25 nucleotides. They are generated by the action of Dicer, an RNase that processes hairpin structured precursors (called pre-miRNA) into mature miRNAs. They post-transcriptionally repress gene expression by recognizing complementary target sites in the 3′untranslated region (UTR) of target mRNAs (Yong and Dutta, 2009). Functionally, the miRNA serves as a guide to the target mRNA through base-pairing and thereby negatively regulating the target expression. The mechanism of silencing, whether through translation inhibition or through cleavage of target mRNA with subsequent degradation is determined by the complementarity level between miRNA and mRNA (Aravindan et al., 2019).

It is estimated that 1–4% genes in the human genome are miRNAs (Esquela-Kerscher and Slack, 2006) and they control the expression of over 60% of the protein-coding genes (Catalanotto, Cogoni and Zardo, 2016). The miRNAs can even be involved in modulating extrinsic factors, such as stromal cell interactions, immune system interactions and even sensitivity to therapy, making these molecules the subject of intense ongoing research (Dobre et al., 2021).

miRNAs are key players, playing important roles in cell proliferation, fate determination, and cell death. Besides these vital processes, miRNAs are implicated in diverse cellular activities, such as immune response (Gantier, Sadler and Williams, 2007), neurotransmitter synthesis (Greco and

Rameshwar, 2007), circadian rhythm, viral replication (Jopling et al., 2005), insulin secretion (Poy et al., 2004), etc. miRNAs are aberrantly expressed in various types of cancers. The first example was reduction of two miRNAs in cancer samples: miR-15a and -16-1 which are clustered at chromosome 13q14, a frequently deleted region in B cell chronic lymphocytic leukemia (CLL) and other cancers (Calin et al., 2002a). miR-15 and miR-16 are frequently deleted in chronic lymphocytic leukemias (Calin et al., 2002b), while reduced levels of miR-Let-7 which targets proto-oncogene RAS (Johnson et al., 2005) and overexpression of polycistronic miR cluster miR-17-92 were shown in lung cancer (Hayashita et al., 2005; O'Donnell et al., 2005).

Role of miRNAs in Neuroblastoma progression and metastasis:

Deregulation of miRNAs may be an important mechanism that leads to pathogenesis and heterogeneity of NB (Schulte et al., 2009; Aravindan et al., 2019). Studies show that under expression of a specific subset of miRNAs in MYCN-amplified NB cells, and targeting MYCN restored these crucial miRNAs that play functional roles (e.g., miR-184 in inducing apoptosis) (Chen and Stallings, 2007; Aravindan et al., 2019). This data suggests that MYCN could induce NB pathogenesis by regulating miRNAs that promote NB cell differentiation, apoptosis, and others (Aravindan et al., 2019).

MYCN binds to the promoter region of an array of miRNAs and regulates their expression. An independent study showed that at least 37 miRNAs were differentially expressed in NB with MYCN amplification compared with MYCN non-amplified NB (Bray et al., 2009). While several oncomiRs (miR-17-5p, miR-92, miR-93, miR-99, miR-106a, and miR-221) are upregulated with MYCN amplification in progressive NB, suppression of tumor suppressors like miR-34a is also observed with MYCN amplification.

In addition, MYCN activates methyl-transferases and prompts methylation of target genes. To that note, deregulated epigenetic machinery can aberrantly modify the promoters of miRNAs in NB (Aravindan et al., 2019). Researchers have identified a panel of oncomiRs and tumor suppressors

(let-7, miR-34a, miR-340, miR-202, miR-101, miR-9, miR-335, and miR-184) that are controlled under epigenetic regulation (aberrant DNA methylation or histone modification) in malignant NB (Romania et al., 2012; Das et al., 2013). Since evolving NB harbors a characteristic miRNA profile and given miRNAs' biomarker designation for NB diagnosis, therapy response, and prognosis (RL, 2009), stabilizing altered miRNA species could improve therapeutic strategies and yield a better outcome (Aravindan et al., 2019).

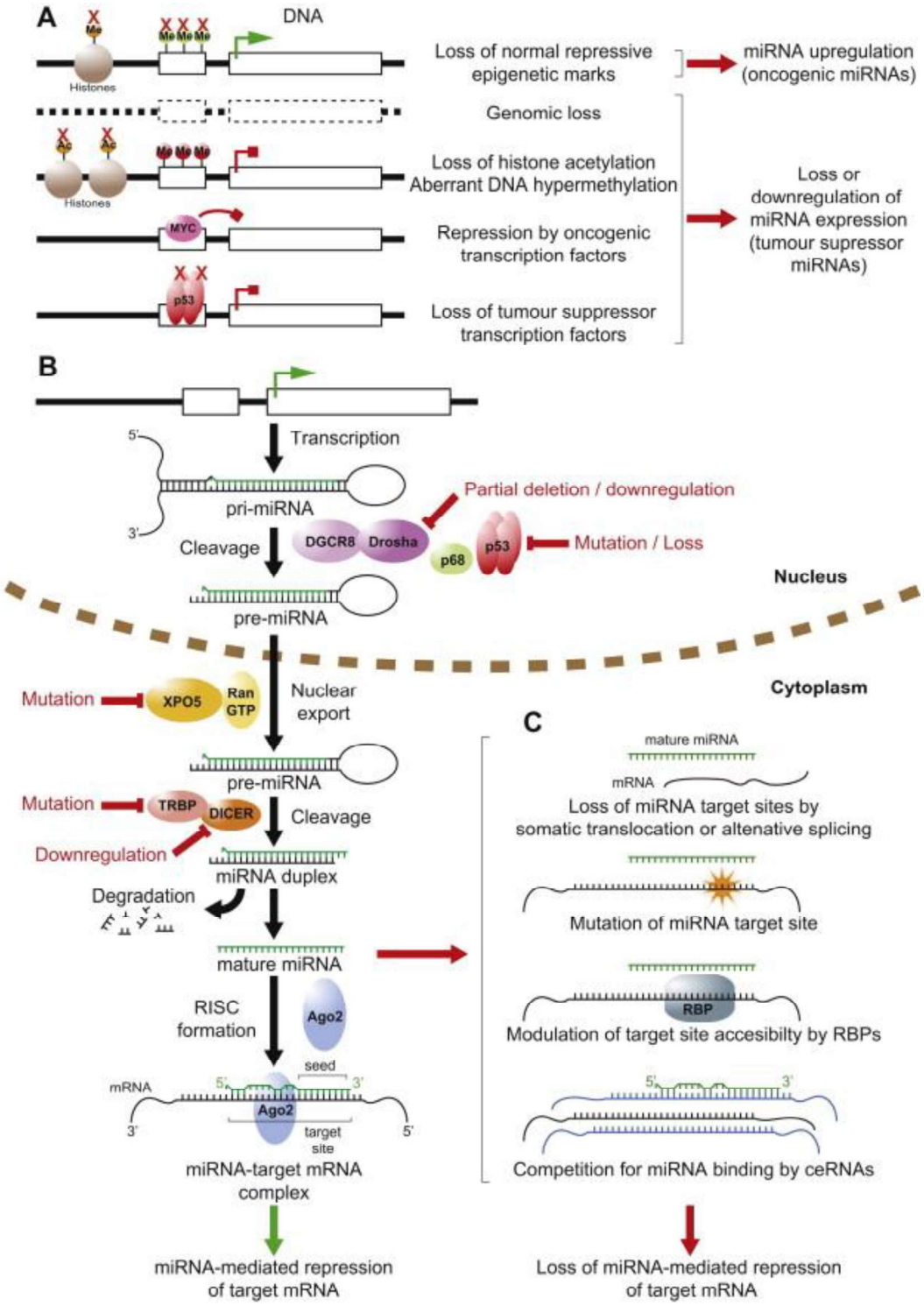

Fig. 1.8: miRNA deregulation in cancer. A schematic representation depicting the canonical miRNA biogenesis pathway and the general mechanisms whereby normal miRNA expression and function can be deregulated in cancer.

Source: (Jansson and Lund, 2012)

Neuroblastoma and treatment strategies:

Considering the high-profile nature of the cancer disease, its treatment has been a continuous hassle with relatively little success. Currently available options for cancer treatment involves surgical removal and radiation treatment of the large accumulated biomass of cancer, which is usually followed by systemic chemotherapy treatment for maintenance. The primarily available chemotherapeutic agents include DNA-interactive agents (e.g., doxorubicin, cisplatin), antimetabolites (e.g., methotrexate), hormones, anti-tubulin agents and molecular targeting agents (Nussbaumer et al., 2011; Choudhari et al., 2019).

High-risk NB patients require treatment with multimodal therapy including induction chemotherapy, surgery, radiotherapy, high-dose chemotherapy with autologous stem cell rescue, and biologic and immunotherapeutic maintenance therapy in order to improve their survival odds. However, a significant number of patients will still relapse and eventually die of disease (London et al., 2005). Therefore, it remains important for researchers to better understand the origins of NB and develop novel treatment strategies for those who are diagnosed with it.

Currently, the standard treatment for high-risk NB includes three treatment blocks—(i) induction, (ii) consolidation, and (iii) maintenance (Pinto et al., 2015). Induction chemotherapy (IC) aims to reduce the tumor by shrinking it and also reducing the risk of metastasis via chemotherapy and surgery. The consolidation block involves the administration of HDC (high dose chemotherapy) accompanied by ASCT (autologous stem cell transplantation) and radiotherapy. Maintenance involves immunotherapy using anti-disialoganglioside (GD2) monoclonal antibody (mAb) with cytokines and differentiation therapy using 11-cis retinol (Coughlan et al., 2017; Smith and Foster, 2018; Zafar, Wang, Liu, Wang, et al., 2021). Approximately half of high-risk patients do not respond to the first-line therapy protocol or relapse in the first 2 years after treatment (Castel et al., 2001; Pearson et al., 2008; London et al., 2011; Zafar, Wang, Liu, Wang, et al., 2021). The outcome for high-risk NB patients is very poor, with a 5-year survival rate of less than 50%

(Berlanga, Cañete and Castel, 2017). In addition, the response to current standard treatments is highly heterogeneous, varying from total regression to the development of multi-drug resistance and severe toxicities (Zafar, Wang, Liu, Wang, et al., 2021).

The major disadvantages of chemotherapy are recurrence of cancer, drug resistance, and toxic effects on non-targeted tissues that can restrain the use of anticancer drugs and thus impair patient's quality of life. IGF-IR expression is more in children, required for their proper growth and it decreases with age. Blocking IGF-IR for cancer treatment, effects normal tissues as well, leading to diminished quality of life. To overcome the problems of present therapy, search for new promising anticancer agents with better efficacy and lesser side effects continues (Choudhari et al., 2019).

Phytochemicals and derivatives present in plants are promising candidates to improve treatment efficiency in cancer patients and decrease adverse outcomes. A number of these phytochemicals are naturally occurring biologically active compounds with significant antitumor potential. The development of effective and side-effects free phytochemical based anticancer therapy begins with the testing of natural extracts (from dry/wet plant material) for potential anticancer biological activity followed by purification of active phytochemicals based on bioassay-guided fractionation and testing for in vitro and in vivo effects (Choudhari et al., 2019). Almost, 50% of approved anticancer drugs from 1940 to 2014 originated from natural products or directly derived from them (Newman and Cragg, 2016; Choudhari et al., 2019). These phytochemicals have been tested for anti-cancer efficacy at both in vitro and in vivo levels. They possess complementary and overlapping mechanisms to slow down the carcinogenic process by scavenging free radicals (Lee, Huang and Shyur, 2013), suppressing survival and proliferation of malignant cells, as well as diminishing invasiveness and angiogenesis of tumors. They exert wide and complex range of actions on different molecular targets and signal transduction pathways including membrane receptors (Deng et al., 2017), transcriptional factors (Zhang et al., 2017), downstream tumor-

activator or -suppressor proteins (Adams et al., 2010), kinases (Dou et al., 2018), cyclins, and caspases (Zhao et al., 2017) and microRNAs (Petric et al., 2015).

Based on studies showing that the IGF-IR signaling is important for cancer cell growth and survival, and that it is often overexpressed in malignant and premalignant tissues, many cancers are being treated by IGF-IR inhibitors. Inhibition of tumor growth in vivo has been achieved with anti-IGF-IR antibodies, anti-ligand antibodies, receptor-specific tyrosine kinase inhibitors, and agents such as the Picropodophyllotoxin (PPP) (Waraky, Akopyan, Parrow, Strömberg, Axelson, Abrahmsén, Lindqvist, Larsson and Aleem, 2014).

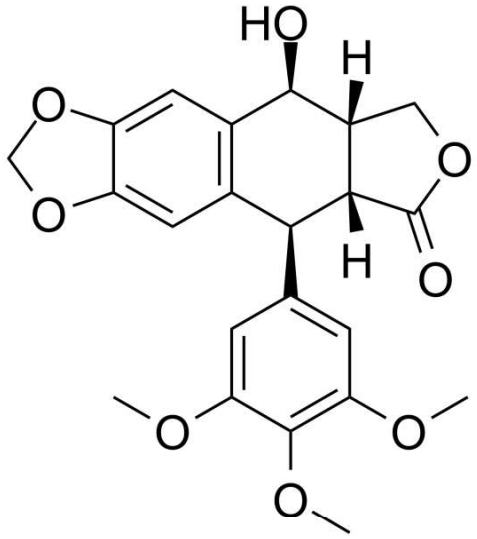

Fig. 1.9: Structure of Picropodophyllotoxin

Picropodophyllotoxin (PPP), a cyclolignan, is a known IGF-IR inhibitor, with a molecular weight of 414.41 and its IUPAC name is (5R,5aR,8aS,9R)-5-hydroxy-9-(3,4,5-trimethoxyphenyl)-5a,6,8a,9-tetrahydro-5H-[2]benzofuro[5,6-f][1,3]benzodioxol-8-one (PubChem). PPP specifically inhibits the activity and downregulates the cellular expression of IGF-IR without interfering with activities of other growth factor receptors, such as receptors for insulin, epidermal growth factor, fibroblast growth factor, platelet-derived growth factor, and mast/stem cell growth factor (PubChem). Even though the exact mechanism has not been established, PPP has been

shown to suppress signaling in the IGF-IR pathway, which is manifested in enhanced degradation of the receptor and reduction of phosphorylated IGF-IR, as well as reduction of phosphorylated down-stream signaling proteins.

PPP can inhibit IGF-IR autophosphorylation, thus resulting in inhibition of cell survival and up-regulation of apoptosis (Waraky et al., 2014). Due to its high specificity and low toxicity, PPP has recently received more and more attention. PPP has been shown to have anti-tumor activity in various types of cancers (Girnita et al., 2004a; Menu et al., 2006a; Dong, Du and Lv, 2019). PPP was also able to inhibit phosphorylation of IGF-IR in tumor tissues of xenografted mice and decreased phosphorylation of downstream signaling molecules (Girnita et al., 2004, 2008). Thereafter, the tumor cell xenografts underwent complete regression. Furthermore, PPP can also hamper growth in multiple myeloma cell lines, bone marrow stromal cells (Scagliotti & Novello, 2012), uveal melanoma cells (Vasilcanu et al., 2006a), 5T33MM mouse model (Menu et al., 2006b) and colon cancer cells (Feng et al., 2012).

HYPOTHESIS:

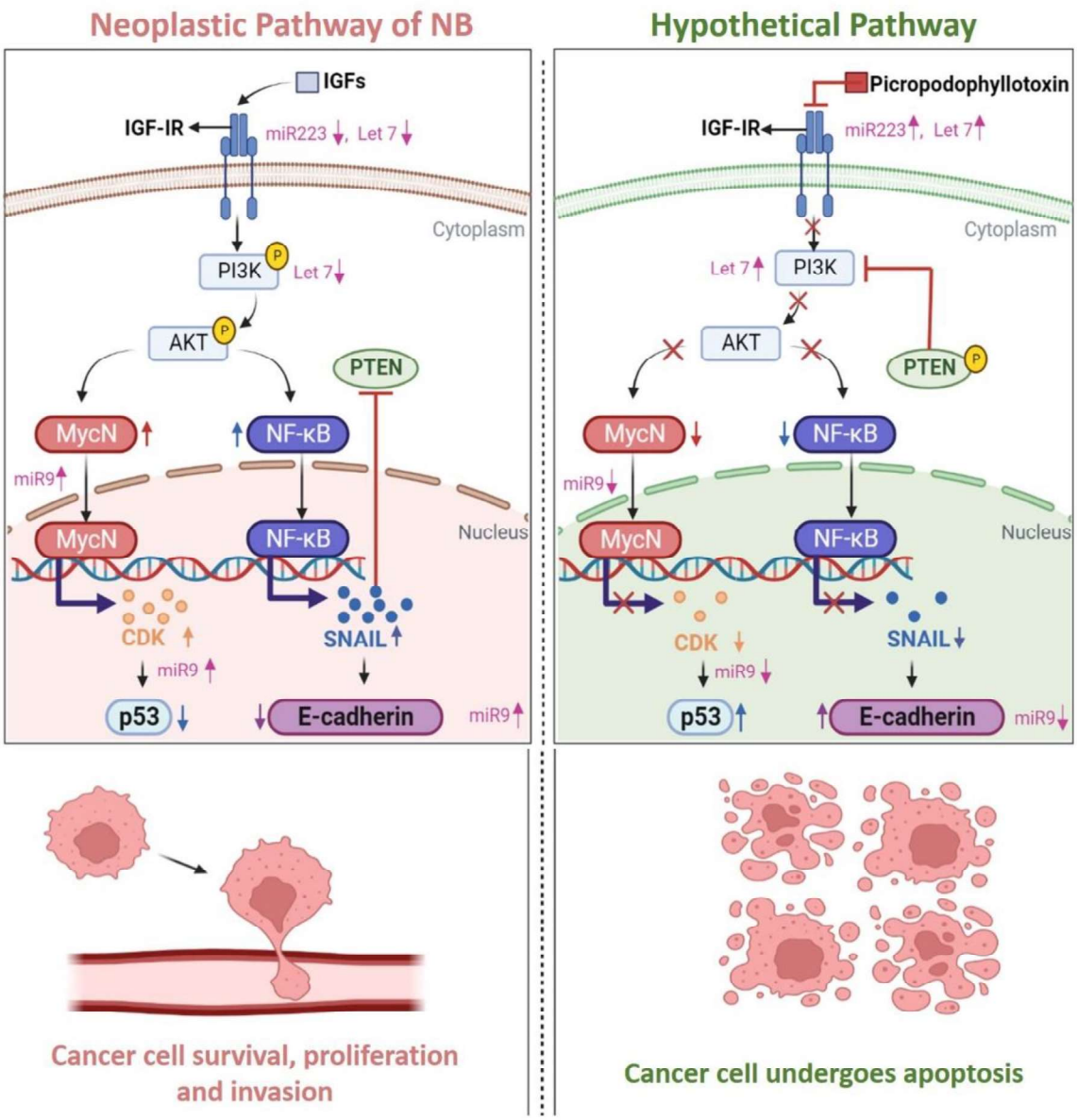

Fig 1.10: Schematic diagram of proposed hypothesis

IGF-IR is one of the oncogenes and its expression is stabilized by crosstalk of other oncogenes and tumor suppressor gene when tumorigenic cascade occurs. miRNAs are found to be the prospective candidate, as it can directly target multitude of genes downstream of IGF-IR pathway, thereby giving less scope for the cancer cells to bypass and activate the alternate signaling pathway. miRNA is differentially expressed in cancer as well as involved in cancer metastasis. Invasion and metastasis of NB may be stabilized by miRNA interaction with IGF IR and kinases. However, the expression pattern and detailed roles of miRNA in NB remains unknown. Here, it is hypothesized that miRNA expression modulates the IGF-IR signaling pathway by inhibiting

IGF-IR expression and consequently plays tumor-suppressing roles in NB progression. Considering the impact of overexpression of IGF-IR in NB, it is an ideal therapeutic target for NB treatment. Our present study is aimed to identify and validate a microRNA which can downregulate IGF1R and its downstream effectors in NB.

OBJECTIVES

The main aim of the current proposal is to unveil the mechanism of IGF-IR inhibition and tumor regression of neuroblastoma to validate the IGF-IR as one of the promising therapeutic targets for NB cancer.

To achieve the above goal following parallel objectives are designed:

a. Comparison of perturbed downstream pathway on IGF-IR suppression.

b. Validation at protein level expression and NB regression.