chapter seven.

general discussion
The concept of the hallmarks of cancer, in particular the biological characteristics acquired during tumor progression, is now a widely appreciated concept. Initially Hanahan and Weinberg postulated six characteristics: sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, activating invasion and metastasis, and inducing angiogenesis (Hanahan, Weinberg 2000). However, over the past decade it has become increasingly apparent that crosstalk between tumor cells and the surrounding stroma greatly promotes tumor progression by affecting most, if not all, hallmarks of cancer (Egeblad, Nakasone & Werb 2010, Joyce, Pollard 2009). Among various possibilities, the secretion of factors that enable adaptive changes in nearby cells in a paracrine fashion seems to underpin the crosstalk between tumor and stromal cells. Such tumor/stromal cell crosstalk is mediated via receptor-ligand interactions and activation of intracellular signal transduction pathways that regulate changes in gene transcription and thereby cell behavior. As outlined in chapter 1 and throughout this thesis, two of such receptor-ligand interactions with major implications for tumor progression are the canonical Frizzled receptor-Wnt and E-type prostanoid (EP) receptor-prostaglandin E$_2$ (PGE$_2$) interactions. The contribution of canonical Wnt and concomitant activation of the primary downstream effector β-catenin, is well documented in a variety of solid tumors (Reya, Clevers 2005). PGE$_2$ is the metabolic product of cyclooxygenase (COX) enzymes, of which the inducible isoform COX-2 is often found increased in malignant tumor cells (Sinicrope, Gill 2004, Liu et al. 2001, Chang et al. 2005, Hida et al. 1998). Binding of PGE$_2$ to the EP2 and EP4 receptor, members of the extensive family of G-protein coupled receptors (GPCRs), induces the accumulation of cyclic AMP (Hull, Ko & Hawcroft 2004). The identification of the cyclic AMP-regulated guanine exchange factors (GEFs), exchange protein directly activated by cyclic AMP 1 (Epac1) and Epac2, as novel cyclic AMP effectors next to protein kinase A (PKA), added further complexity to GPCR and cyclic AMP signaling (de Rooij et al. 1998, Kawasaki et al. 1998).

The objective of this thesis was to establish interactions between cyclic AMP and β-catenin in relation to the hallmarks of cancer. In particular, this thesis aims to investigate the role of PGE$_2$, and activation of the downstream cyclic AMP effectors PKA and Epac on β-catenin and β-catenin-mediated transcription. The studies described in this thesis reveal a critical regulatory role for the cyclic AMP effectors in β-catenin-mediated transcription and tumor progression.

**Prostaglandin E$_2$ and β-catenin team up in cancer**

Observations indicating that PGE$_2$-signaling involves activation of β-catenin-mediated transcription accumulated over the past decade (Castellone et al. 2005, Goessling et al. 2009, Shao et al. 2005, Dohadwala et al. 2006, Ho et al. 2013, Kim et al. 2010, Singh, Katiyar 2013, Zhang et al. 2014). Although these observations solidly established that PGE$_2$ and β-catenin are intimately connected during tumor progression, the studies failed to provide conclusive answers on the mechanisms by which this interaction occurs. There is evidence for the involvement of Gas/Axin complex formation, activation of Phosphoinositide 3-kinase (PI3K)/Akt and subsequent inhibition of glycogen synthase kinase 3 (GSK3) (Castellone et al. 2005), activation of PKA and subsequent inhibition of GSK3 or direct stabilization...
of β-catenin by means of phosphorylation (Shao et al. 2005, Taurin et al. 2006, Hino et al. 2005), and the involvement of β-arrestin/Src complexes (Kim et al. 2010). Chapter 3 describes that downstream of PGE₂, β-catenin is indeed stabilized by PKA through direct phosphorylation, but also through inhibition of GSK3. Although a role for Epac has not been described before, studies on small GTPases indicated that Epac-dependent activation of Rap1 and Rac activates β-catenin-mediated transcription (Goto et al. 2010, Wu et al. 2008). In addition, activation of Rac1 has been recognized as a driving factor in β-catenin stabilization, nuclear import and target gene transcription (Wu et al. 2008). Chapter 4 describes a novel role for Epac1 in driving PGE₂-induced activation of β-catenin-mediated transcription.

**PGE₂, retinoids and β-catenin in neuroblastoma**

One of the most fundamental traits of tumor cells is the ability to sustain continuous proliferation. Tumors achieve this by production of pro-mitotic factors, which elicit an autocrine response (Bhowmick, Neilson & Moses 2004, Lemmon, Schlessinger 2010). Regarding neuroblastoma, the observation that β-catenin expression is increased in a subset of neuroblastomas that are not characterized by amplification of MYCN, an important adverse prognostic marker and driver of proliferation (Liu et al. 2008), provoked the question whether these neuroblastomas are dependent on the increased β-catenin for their survival. Chapter 3 aimed to answer this question and further elaborated on the role of COX-2 and PGE₂ in relation to β-catenin-mediated transcription. The data shown there demonstrate that in MYCN non-amplified neuroblastoma cells, PGE₂ enhances cell viability through the EP4 receptor, a process sensitive to COX-4 inhibition. As both PGE₂ and cyclic AMP elevation, by the direct adenyl cyclase activator forskolin, inhibited GSK3 and phosphorylated β-catenin at PKA target residues, we proposed that the effects of PGE₂ on β-catenin are mediated through activation of PKA. Furthermore, inhibition of β-catenin using the XAV939 prevented the effects of PGE₂. Inhibition of COX-2, and thus attenuation of PGE₂ synthesis, resulted in both an increase in apoptotic events and a decrease in cell cycle progression. In chapter 3, increased expression of β-catenin in high-risk neuroblastoma was observed in tumors without MYCN amplification, but not in low-risk tumors. Indeed, other recent studies indicated that β-catenin promotes a neuroblast-like, non-differentiated, phenotype of neuroblastoma cells which is highly resistant to therapy (Vangipuram, Buck & Lyman 2012, Zhi et al. 2012). Together this suggest that β-catenin is plays a critical role in proliferation of neuroblastoma cells (Fig. 1).

Therapeutically, retinoids, in particular all-trans retinoic acid, are used to induce an arrest of cell growth and morphological differentiation in high-risk neuroblastoma (Sidell 1982, Reynolds et al. 2003). Retinoic acid functions by binding to its nuclear receptors, retinoic acid receptor (RAR) and retinoid X receptor (RXR), which together form a complex that regulates transcription of target genes. However, compelling evidence indicate that retinoids inhibit β-catenin-mediated transcription by inducing proteasomal degradation of β-catenin (Park et al. 2005, Easwaran et al. 1999, Dillard, Lane 2008, Dillard, Lane 2007, Xiao et al. 2003, Han et al. 2008, Benelli et al. 2010). Indeed, in chapter 6 all-trans retinoic acid decreased β-catenin in neuroblastoma, a process accompanied by an increase in Epac1
Model of the role of PGE$_2$-induced β-catenin activation in relation to regulating cell proliferation and differentiation in neuroblastoma. Chapter 3 demonstrates that PGE$_2$ enhances neuroblastoma cell proliferation through cyclic AMP-mediated activation of PKA. PKA, in turn, activates β-catenin, either via direct phosphorylation, or via inhibition of GSK3. The resulting stabilized β-catenin translocates to the nucleus where it activates transcriptional responses via association and activation of TCF. In this way, PGE$_2$ and β-catenin drive cell proliferation and inhibit differentiation of neuroblast-like neuroblastoma cells. All-trans retinoic acid (atRA) is used therapeutically to induce neuroblastoma cell differentiation. atRA associates with and activates the RAR and RXR transcription factors. Observations from other groups showing that atRA inhibits COX-2 expression and thereby PGE$_2$ synthesis, in combination with our observation in Chapter 6 showing that atRA inhibits β-catenin activity, indicate that atRA can counteract the aforementioned differentiation-inhibiting PGE$_2$-β-catenin interaction.
and Epac2 gene expression. Importantly, pharmacological inhibition of Epac2 led to a morphology resembling differentiation to S-type neuroblastoma characterized by flattened, highly adherent cells that resemble Schwann cells and by lower malignancy (Spengler et al. 1997). In addition, inhibition of the interaction between β-catenin and cyclic AMP response element-binding protein (CREB)-binding protein (CBP) by ICG001 induced differentiation of neuroblastoma cells and all-trans retinoic acid-induced differentiation was potentiated by inhibition of β-catenin/CBP, indicating that β-catenin/CBP-mediated transcription is involved in keeping neuroblastoma cells in the neuroblast-like phenotype (Vangipuram, Buck & Lyman 2012, Zhi et al. 2012).

Interestingly, suppression of COX-2 by retinoids has been demonstrated in a variety of carcinomas (Merritt et al. 2001, Mestre et al. 1997a, Mestre et al. 1997b, Subbaramaiah, Cole & Dannenberg 2002, Kanekura, Higashi & Kanzaki 2000). Mechanistically, APC, the β-catenin destruction complex component which often has a loss of function mutation in cancer, controls the biosynthesis of all-trans retinoic acid (Eisinger et al. 2006). As such, the lack of a functional APC protein correlates with elevated expression of COX-2, which was attenuated by treatment with all-trans retinoic acid. Further, COX-2 inhibition enhanced the growth inhibitory effects of all-trans retinoic acid in colorectal carcinoma (Liu et al. 2010), and all-trans retinoic acid treatment of zebrafish embryos and colorectal carcinoma cells decreased the levels of β-catenin by a mechanism that requires the attenuation of COX-2 expression, and the subsequent decrease in PGE\(_2\) (Eisinger et al. 2007). Thus, the data indicate that the decreased levels of β-catenin by all-trans retinoic acid treatment are most likely the consequence of attenuation of COX-2 by all-trans retinoic acid.

**PGE\(_2\) and β-catenin in epithelial-mesenchymal transition**

During acquisition of traits that allow for local invasion and distant metastasis, carcinoma cells typically developed alterations in shape and in their attachment to other cells and the ECM. The best characterized alteration involves the loss of the adherens junction protein E-cadherin. Epithelial-to-mesenchymal transition (EMT) has become the predominant paradigm by which carcinoma cells acquire the abilities to invade and to disseminate and is orchestrated by a set of pleiotropically actin transcription factors (Thiery et al. 2009, Polyak, Weinberg 2009).

In chapter 4 the effects of PGE\(_2\) on β-catenin stabilization and activation of β-catenin-mediated transcription are studied in relation to EMT in non-small cell lung carcinoma.

EMT can be induced by a variety of growth factors and other molecular mediators, including PGE\(_2\) via a mechanism requiring stabilization of β-catenin and activation of β-catenin-dependent transcription (Dohadwala et al. 2006, Ho et al. 2013, Kim et al. 2010, Singh, Katiyar 2013, Zhang et al. 2014). During EMT, β-catenin has a dual function: on one hand it complexes with E-cadherin at the adherens junction, while on the other hand, nuclear β-catenin acts as a transcriptional co-regulator of genes involved in metastasis. Thus, activation of β-catenin-mediated transcription induces EMT, thereby down-regulating E-cadherin expression, further releasing β-catenin form the adherens junction thereby creating a positive feedback loop that attenuates cell-cell adhesion and reinforces EMT (Mukherjee et al. 2014).
**Chapter 4** describes that PGE2 induced downregulation of E-cadherin via activation of β-catenin. EMT is tightly regulated through several families of transcription factors, including ZEB1. PGE$_2$ has been shown to increase gene expression of ZEB1 (Dohadwala et al. 2006, Ho et al. 2013) which is in line with the findings discussed in **chapter 4**. Importantly, expression of Epac1 was increased by PGE$_2$. Using both RNAi and selective pharmacological inhibition of Epac1, **chapter 4** demonstrates that Epac1 is required for PGE$_2$-induced nuclear localization of β-catenin, β-catenin-mediated transcription and transcription of ZEB1.

**PGE$_2$ and β-catenin in hypoxia and hypoxia-driven EMT**

Tumors require nutrients and oxygen as well as the ability to dispose metabolic waste. The tumor-associated neovasculature, generated through angiogenesis, allows tumors to cope with these needs. The lack of sufficient oxygen supply, hypoxia, triggers as series of adaptations inside tumor cells that drive cellular adaptations to the perceived hypoxia, such as the release of growth factors that stimulate the formation of new vasculature. In addition, inside the tumor, hypoxia drives EMT (Gilkes, Semenza & Wirtz 2014) and it appears that the factor that initiates production of pro-angiogenic growth factors, hypoxia inducible factor 1α (Hif1α), also underlies hypoxia-driven EMT (Sahlgren et al. 2008, Krishnamachary et al. 2003). Interestingly, studies have revealed that the metastasis promoting effect of β-catenin is absent upon Hif1α silencing, indicating an interaction between β-catenin and Hif1α (Santoyo-Ramos et al. 2014, Zhang et al. 2013). **Chapter 5** demonstrates that hypoxia inhibits β-catenin/TCF-mediated transcription in colorectal carcinoma cells. Hypoxia did not only inhibit basal β-catenin/TCF-mediated transcription, but also its induction by the canonical Wnt ligand Wnt3a, indicating that hypoxia effectively inhibits canonical Wnt/β-catenin activation. Earlier studies from colleagues found that hypoxia inhibited the β-catenin/TCF complex formation, and as a result, β-catenin transcriptional activity (Kaidi, Williams & Paraskeva 2007). This study demonstrated that both transcription factors, Hif1α and TCF, compete for direct binding of β-catenin. The interaction between Hif1α and β-catenin occurs at HREs and as such, β-catenin enhances Hif1α-mediated gene transcription, promoting cellular adaptation to hypoxia. Taking into account the findings discussed in **chapter 3** and **chapter 4** that show PGE$_2$-induced activation of β-catenin transcriptional activity, studies on PGE$_2$ in hypoxia are of particular interest. Early studies in colorectal carcinoma cells demonstrated that overexpression of COX-2 promotes the production of angiogenic factors, such as VEGF, and stimulates endothelial cell proliferation, migration and vascular tube formation, effects inhibited by non-steroid anti-inflammatory drugs (NSAIDs) (Tsujii et al. 1998). The inhibitory effect of NSAIDs on angiogenesis in this context can be rescued by adding exogenous PGE$_2$, suggesting that COX-2-derived PGE$_2$ is at least part responsible for the pro-angiogenic effects of COX-2 overexpression (Jones et al. 1999). Consistent with this, PGE$_2$ has been reported to stimulate VEGF expression through the activation of Hif1α (Fukuda, Kelly & Semenza 2003), an effect that is likely mediated through the EP2 receptor as mice that are null for the EP2 receptor lack PGE$_2$-induced VEGF expression (Sonoshita et al. 2001). On the other hand, expression of COX-2 is regulated by Hif1α through Hif1α binding.
to the HRE elements in the COX-2 promoter region leading to enhanced PGE$_2$ levels and PGE$_2$-mediated vasculatization (Csiki et al. 2006, Kaidi et al. 2006), a mechanism that appears to be not exclusive for carcinoma as it also appears in human vascular endothelial cells (Schmedtje et al. 1997).

Chapter 4 reports on PGE$_2$-induced nuclear accumulation of β-catenin and β-catenin-mediated transcription, and points to a pivotal role of Epac1 in nuclear translocation and activation of β-catenin/TCF-mediated transcription. Therefore, in chapter 5 the role of Epac in determining the outcome of hypoxia-driven inhibition of β-catenin/TCF-mediated and activation of Hif1α-mediated transcription is studied. We observed a marked increase in mRNA expression of Epac2 in colorectal carcinoma cells. Pharmacological inhibition of Epac2 completely abolished Wnt3a-induced activation of β-catenin/TCF-mediated transcription, indicating that Epac2 plays a role in the canonical Wnt/β-catenin pathway in colorectal carcinoma cells. Importantly, pharmacological inhibition of Epac2 is accompanied by an increased expression of the Hif1α target gene VEGFA and VEGFA secretion from the colorectal carcinoma cells. Together, our data suggests a novel role for Epac2 in mediating β-catenin/TCF-mediated transcription, while blocking Hif1α-mediated transcription in hypoxic colorectal carcinoma cells. Together, the findings outlined above demonstrate PGE$_2$-induced activation of β-catenin in normoxia (chapter 3, chapter 4), PGE$_2$-induced activation of Hif1α/VEGF in hypoxia (Fukuda, Kelly & Semenza 2003, Kaidi et al. 2006), and β-catenin switching between TCF and Hif1α in normoxia and hypoxia, respectively (chapter 5). These findings suggest on the one hand, a selective role for PGE$_2$ in the stimulation of cell proliferation that is restricted to normoxia, and on the other hand that the proliferative effect of PGE$_2$ may be overridden as a result of Hif1α competing with TCF for β-catenin in hypoxia. Such scenario may represent novel roles for Epac1 and Epac2 in regulating this spatial-temporal switching in PGE$_2$ signaling to β-catenin between normoxia and hypoxia (Fig. 2).

**Compartmentalized interaction of cyclic AMP and β-catenin**

There are at least two pools of β-catenin intracellularly, one pool at the cell membrane leading to the association of β-catenin to the adherens junctions, and one pool in the cytosol. The cytosolic pool has a very short life-span as it is constantly tagged for degradation by the β-catenin destruction complex, through sequential phosphorylation by GSK3 and casein kinase 1 (CK1) and subsequent ubiquitination and proteosomal degradation (Clevers, Nusse 2012). As discussed before, stabilization of the cytosolic pool of β-catenin, for example through canonical Wnt signaling or PGE$_2$, allows β-catenin to translocate to the nucleus thereby enabling it to function as a transcriptional co-factor. During EMT, carcinoma cells lose their epithelial polarity and show a downregulation of E-cadherin and concomitant dissociation of the adherens junction (Berx, van Roy 2009). Besides E-cadherin, the adherens junction consists of several catenin family members, most notably β-catenin. Thus, loss of E-cadherin induces dissociation of β-catenin from the junctional complex, and thereby increases the cytosolic pool, which thereby becomes available for stabilization, downstream of canonical Wnt signaling or PGE$_2$. Chapter 4 investigates the role of Epac1 in PGE$_2$-induced EMT in relation to β-catenin.
Although this chapter proposes a role for Epac1 in relation to the cytosolic pool of β-catenin, chapter 2 outlines mechanisms by which Epac1 could also regulate the junctional pool. PGE$_2$ has been shown to activate Rac1 through the GEF activity of Epac1 in endothelial cells (Birukova et al. 2007, Birukova et al. 2010, Maillet et al. 2003) and crosstalk between Epac1 and Rac1 was observed in cervical carcinoma and fibrosarcoma stimulating cell migration (Harper et al. 2010, Lee, Lee & Moon 2014). IQ-motif containing GTPase activating protein 1 (IQGAP1) is an Rac1 effector that exhibits a high expression level with close correlation to metastatic potential. IQGAP1 binds to E-cadherin and β-catenin and stabilizes their interaction and thereby the junctional complex (Nabeshima et al. 2002, Jadeski et al. 2008, Nakamura et al. 2005). Activated Rac1, for example by PGE$_2$/Epac1, binds to IQGAP1 and thereby dissociates IQGAP1 from the adherens junction (Hage et al. 2009). Such events result in altered subcellular distribution of E-cadherin and β-catenin, with β-catenin dissociating from E-cadherin. Importantly, activation of Rac1 has also been recognized as a driving factor in β-catenin stabilization, nuclear import and target gene transcription (Wu et al. 2008) and recent studies on the Rac1 effector kinase p21-activated kinase (PAK1) showed that PAK1 stabilizes β-catenin by phosphorylation (Zhu et al. 2012, Arias-Romero et al. 2013). Thus, crosstalk between Epac1 and Rac1 may result in decreased junctional stability and increased stability of free cytosolic β-catenin.

Once stabilized, cytosolic β-catenin enters the nucleus. In this regard, the finding that Epac1 directly binds to the nuclear pore protein RanBP2 at the nuclear membrane through its CDC25-HD domain is particularly of interest (Liu et al. 2010, Gloerich et al. 2011, Parnell, Smith & Yarwood 2015, Qiao et al. 2002). In chapter 4, we showed that PGE$_2$-induced activation of β-catenin-dependent transcription was abolished in non-small cell lung carcinoma cells expressing an Epac1 mutant that lacks the Ran binding protein 2 (RanBP2) binding sequence, indicating that the association between RanBP2 and Epac1 is required for this PGE$_2$-mediated effect on β-catenin. In addition, this chapter shows that Epac1 associates with β-catenin, through the common adaptor protein Ezrin (Gloerich et al. 2010, Hiscox, Jiang 1999). Ezrin belongs to the Ezrin-radixin-moesin family of A-kinase anchoring proteins (AKAPs). AKAPs play a very prominent and important role in cyclic AMP signaling, as these anchoring proteins associate with receptors, kinases, phosphatases, PDEs, and ion channels and thereby are able to regulate the spatio-temporal signal specificity of cyclic AMP signaling (Poppinga et al. 2014). Interestingly, expression of Ezrin correlates with an invasive phenotype in several carcinomas, and knockdown of Ezrin reduces migration and invasion of carcinoma cells (Saito et al. 2013, Li et al. 2012, Li et al. 2008, Gavert et al. 2010). In chapter 4, we reported that knockdown of Ezrin using RNAi strongly reduced PGE$_2$-induced β-catenin-dependent transcription. In addition, the association between Epac1 and β-catenin is lost upon Ezrin knock-down, indicating that Ezrin is required for the Epac1 and β-catenin association in non-small cell lung carcinoma cells. Interestingly, Ezrin is phosphorylated by PKA at T567, thereby promoting an open conformation enabling interaction with β-catenin and Epac1 (Zhu et al. 2007, Parnell et al. 2015). Thus, activation of PKA by PGE$_2$, downstream of the Gs-coupled EP receptors (chapter 3) potentially activates Ezrin directly.

Together our data and evidence in the literature suggest that PGE$_2$, and the subsequent cyclic
β-Catenin-mediated transcription in normoxia and hypoxia. In hypoxia, nuclear β-catenin can associate with Hif1α, thereby dissociating from TCF. Chapter 5 describes that in hypoxia, Epac2 expression is increased. Inhibition of Epac2 results in increased expression of Hif1α target genes, while this attenuates β-catenin target gene transcription, suggesting that Epac2 expression in hypoxia may function to restore the balance between β-catenin/TCF and β-catenin/Hif1α. In addition, hypoxia induces COX-2 expression and thereby enhances PGE2 levels. Chapter 3 and chapter 4 describe that PGE2 can stabilize β-catenin and activate β-catenin-mediated transcription in a cyclic AMP-dependent manner. Chapter 3 demonstrates that upon PGE2, PKA phosphorylates and stabilizes β-catenin, while chapter 4 demonstrates that Epac1 associates with β-catenin at the nuclear pore and is required for β-catenin/TCF-mediated transcription. Together this indicates that the cyclic AMP effectors Epac1, Epac2 and PKA are involved in β-catenin/TCF-mediated transcription downstream of PGE2.
AMP spike, activates β-catenin-mediated transcription by the orchestrated actions of the cyclic AMP effectors Epac and PKA (Fig 3).

**Main conclusions**

- In addition to the canonical Wnt pathway, β-catenin also functions downstream of PGE₂ in several malignant cell types, including neuroblastoma and non-small cell lung carcinoma cells (Chapters 3 and 4).
- PGE₂ activates β-catenin in a cyclic AMP-dependent manner, and involves both cyclic AMP effects, PKA and Epac1 (Chapters 3 and 4).
- In neuroblastoma, β-catenin and PGE₂-induced β-catenin activation drive an aggressive phenotype in a subset of high-risk tumors that are not characterized by amplification of MYCN. β-Catenin/CBP-mediated transcription keeps neuroblastoma cells in a neuroblast-like phenotype. All-trans retinoic acid inhibits β-catenin/CBP-mediated transcription and induces differentiation (Chapters 3 and 6). This suggests that β-catenin is an interesting therapeutic target for the treatment of high-risk neuroblastoma without MYCN amplification.
- Inhibition of Epac2 in combination with all-trans retinoic acid potentiates all-trans retinoic acid-induced differentiation towards S-type cells with low malignancy (Chapter 6). This suggests that a combinatorial approach with all-trans retinoic acid and a selective Epac2 subtype inhibitor may have promise for the treatment of high-risk neuroblastoma.
- Hypoxia shift β-catenin/TCF-mediated transcription towards β-catenin/Hif1α-mediated transcription in several malignant cell lines (Chapter 5). In hypoxia, Epac2 inhibition potentiates expression of Hif1α target genes, while inhibiting β-catenin/TCF target genes in colorectal carcinoma cells (Chapter 5). This implies that Epac2 is an important modulator of the hypoxic response in colorectal carcinoma cells.
- PGE2-induced epithelial-to-mesenchymal transition in non-small cell lung carcinoma cell involves Epac1 and subsequent β-catenin-mediated transcription (Chapter 3).
- Epac1 and β-catenin associate together through the common scaffold protein Ezrin. This association is crucial for PGE2-induced β-catenin-mediated transcription in non-small cell lung carcinoma cells (Chapter 3).
Future Perspectives

The studies described in this thesis show that PGE₂, through the cyclic AMP effectors PKA and Epac, affects β-catenin-mediated transcription. Although a role for PKA in regulation of β-catenin stability is known, a role for Epac in relation to β-catenin has not been described before. Although the subtype of Epac involved differs depending on the cell type and on the physiological context, in general interaction between Epac and β-catenin appears to favor β-catenin-mediated transcription. Over the past years, a role for Epac in cancer is emerging (Almahariq, Mei & Cheng 2015). Therefore, these findings shine a new light on the therapeutic potential of drugs that interfere with PGE₂ (e.g. NSAIDs) and β-catenin in the context of cancer therapeutics. This thesis has only focused on the role of these interactions in cancer cells. However, tumors are more than insular masses of proliferating cancer cells. Instead, tumors are complex tissues composed of distinct cell types. In addition, this thesis has not explored whether the described interactions between cyclic AMP effectors and β-catenin also take place downstream of other cyclic AMP coupled receptors besides the EP receptors. As such, studies focusing on the interaction between cyclic AMP and β-catenin in different cell types and focusing on different receptors would be of great interest. For example, catecholamines bind to and activate adrenoceptors, such as the β₂-adrenoceptor, which are a family of GPCRs. Just as NSAIDs, antagonists of β-ARs, β-blockers, have been one of the most widely used drugs for the last half century. Interestingly, observations indicating the potential for their use as anti-cancer drugs are beginning to emerge retrospective case control studies and pre-clinical in vitro and in vivo studies. As such, the use of β-blockers could have preventive and protective effects in several malignancies (Fitzgerald 2010, Cole et al. 2015, Cole, Sood 2012). In addition, several lines of research have found that β₂-AR signaling induces the expression of pro-angiogenic factors, such as VEGF, in tumor cells (Thaker et al. 2006, Yang et al. 2006, Lee et al. 2009). Studies in xenograft models have found that only the combined use of β-blockers and inhibition of COX-2, the enzyme responsible for synthesis of PGE₂, was effective in preventing catecholamine tumor angiogenesis (Lee et al. 2009, Neeman, Zmora & Ben-Eliyahu 2012, Horowitz et al. 2015). Importantly, a recent study found that the effects of β₂-AR signaling in tumor progression are mediated via activation of the COX-2/PGE₂ pathway (Nagaraja et al. 2015). This thesis, together with the findings of the aforementioned studies, suggests that cyclic AMP and β-catenin play a potential important role, not only downstream of the PGE₂ receptors, but also of other Gαs-coupled receptors such as the β₂-AR.
There are at least two pools of β-catenin intracellularly: one at the adherens junctions and one cytosol. Stabilization of the cytosolic pool of β-catenin allows β-catenin to translocate to the nucleus and activate target gene transcription through association with transcriptional co-activators, such as TCF. In Chapter 2 we describe a model in which the GTPase Rac1 is activated in an Epac-dependent manner. The resulting Rac1 activation leads to destabilization of the interaction between the Rac effector IQGAP1 and β-catenin at the adherens junction, thereby dissociating β-catenin from the adherens junction. In addition, active Rac1 can also activate β-catenin-mediated target gene transcription by facilitating β-catenin nuclear import. Chapter 3 demonstrates that PGE₂ activates β-catenin and β-catenin-mediated transcription, either via direct phosphorylation, or via inhibition of GSK3. Chapter 4 demonstrated that Epac1 is required for PGE₂-induced β-catenin-mediated transcription. Epac1 and β-catenin associate together through the common adaptor protein Ezrin. The association between β-catenin and Epac1 is lost when an Epac1 sequence responsible for association with the nuclear pore protein RanBP2 is lost, suggesting that the interaction occurs at the nuclear pore complex. Together these observations describe that PGE₂ activates β-catenin-mediated transcription by the orchestrated actions of the cyclic AMP effectors Epac and PKA.


Chapter seven


References

M - S


Chapter seven

S

Z


