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# P-cadherin role in normal breast development and cancer

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**ABSTRACT P-cadherin is a cell-cell adhesion molecule, whose expression is highly associated with undifferentiated cells in normal adult epithelial tissues, as well as with poorly differentiated carcinomas. Its expression has been already reported in human embryonic stem cells and it is presumed to be a marker of stem or progenitor cells of some epithelial tissues. In normal breast, P-cadherin has an essential role during ductal mammary branching, being expressed by the monolayer of epithelial cap cells at the end buds. In mature mammary tissue, its expression is restricted to the myoepithelium; it has been postulated that it may also be present in early luminal progenitor cells. In breast cancer, P-cadherin is frequently overexpressed in high-grade tumours, being a well-established indicator of poor patient prognosis. It has been reported as an important inducer of cancer cell migration and invasion, with underlying molecular mechanisms involving the signalling mediated by its juxtamembrane domain, the secretion of matrix metalloproteases to the extracellular media, and the cleavage of a P-cadherin soluble form with pro-invasive activity. Intracellularly, this protein interferes with the endogenous cadherin/catenin complex, inducing p120-catenin delocalization to the cytoplasm, and the consequent activation of Rac1/Cdc42 and associated alterations in the actin cytoskeleton. Considering P-cadherin's role in cancer cell invasion and metastasis formation, a humanized monoclonal antibody was recently produced to antagonize P-cadherin-associated signalling pathways, which is currently under Phase I clinical trials. In this review, the most important findings about the role of P-cadherin in normal breast development and cancer will be illustrated and discussed, with emphasis on the most recent data.**

KEY WORDS: *P-cadherin, CDH3 gene, mammary gland, breast cancer*

## Introduction

Classical cadherins constitute a family of molecules that mediate calcium-dependent cell-cell adhesion, localized to the adherens-type junctions. The intracellular domains of cadherins bind directly to cytoplasmic catenins, which link them with the actin cytoskeleton, providing the molecular basis for stable cell interactions. The cadherin/catenin complex, as well as the signalling pathways controlled by this structure, represent a major regulatory mechanism that guide cell fate decisions, through its influence on cell growth, differentiation, motility, and survival (Cavallaro and Dejana, 2011).

Classical cadherins include *CDH1*/E-cadherin (epithelial), *CDH2*/N-cadherin (neuronal), *CDH3*/P-cadherin (placental) and *CDH4*/R-cadherin (retinal), designated by their tissue distribution. E-cadherin is the predominant cadherin family member expressed in all epithelial tissues, being extremely important to the maintenance of the cell shape and polarity; in fact, it is well known that *CDH1* acts as a tumour suppressor gene, negatively regulating the invasion and metastasis of tumour cells in several malignancies (Yilmaz and Christofori, 2010). In contrast, N-cadherin is up-regulated in several cancers and contributes to an invasive phenotype by interacting with fibroblast growth factor receptor (FGFR) and its

*Abbreviations used in this paper:* α–ctn, α-catenin; βctn, β-catenin; CBD, catenin-binding domain; CDH, cadherin; C/EBPβ, CCAAT/enhancer-binding protein β; CK, cytokeratin; CSC, cancer stem cell; CTC, circulating tumour cell; E-cad, E-cadherin; EC, epithelial cell; EEM, ectodermal dysplasia, ectrodactyly, and macular dystrophy; EGFR, epidermal growth factor receptor; ER, estrogen receptor; EMT, epithelial-to-mesenchymal transition; FGFR, fibroblast growth factor receptor; HDAC, histone deacetylase; HJMD, hypotrichosis with juvenile macular dystrophy; IBC, inflammatory breast cancer; JMD, juxtamembrane domain; MECs, myoepithelial cells; MFE, mammosphere forming efficiency; NAF, nipple aspirate fluid; P-cad, P-cadherin; PgR, progesterone receptor; p120ctn, p120-catenin; SHFM, split hand/foot malformation; sP-cad, soluble P-cadherin; TEB, terminal end buds; TSA, trichostatin A.

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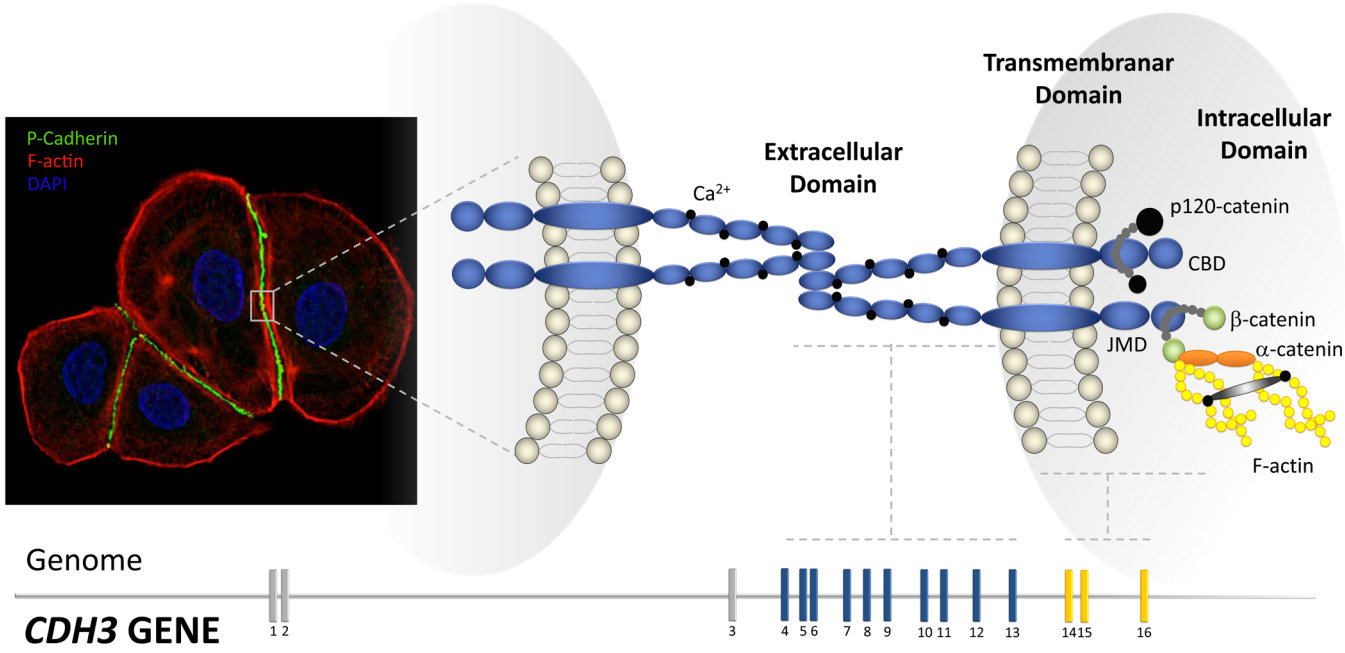


**Fig. 1. Schematic representation of the structural components of the P-cadherin adhesive junction.** *Lateral clustering of P-cadherin molecules is required to form stable cell-to-cell contacts in BT-20 breast cancer cells [immunofluorescence: P-cadherin (green), F-actin (red), DAPI (blue)]. In the intercellular space, P-cadherin extracellular domains interact with P-cadherin extracellular domains of adjacent cells to mediate cell adhesion. The intracellular catenins bind to the cytoplasmic tail of P-cadherin. p120-catenin binds the cadherin tail at the juxtamembrane domain (JMD), whereas* β*-catenin binds to the distal catenin binding domain (CBD).* α*-catenin associates with* β*-catenin and is directly linked to the actin cytoskeleton. The lower panel shows the genomic structure of* CDH3*/P-cadherin gene, which is constituted by 16 exons: the extracellular part of P-cadherin is encoded by 10 exons (exons 4-13), whereas the transmembrane and intracellular domains are determined only by the information included in the last 3 exons (exons 14-16).*

downstream signalling (Suyama *et al.*, 2002).

P-cadherin is also often reported to correlate with increased tumour cell motility and invasiveness when overexpressed (Cheung *et al.*, 2010, Paredes *et al.*, 2004, Ribeiro *et al.*, 2010, Taniuchi *et al.*, 2005). Although the role of P-cadherin encoding gene (*CDH3*) in cancer is far less well characterized than the one attributed to *CDH1*, the opposite effects in mammary cancer are weird, since these molecules share more than 67% of homology (Hulpiau and van Roy, 2009). The *CDH3* gene harbours 16 exons (Fig. 1) and maps to chromosome 16q22.1, a region that contains a cluster of several cadherin genes, just 32 kilobases upstream of the gene encoding human E-cadherin (Bussemakers *et al.*, 1994). The mature P-cadherin glycoprotein structure is similar to that of classical cadherins, comprising three distinct domains (extracellular, transmembrane and intracellular), in order to promote homotypic interactions. At the cell membrane, these create lateral dimers that act together in a zipper-like structure between neighbouring cells (Shapiro *et al.*, 1995) (Fig. 1).

The function and strength of P-cadherin-mediated adhesion depends on its dynamic association with catenins, which link the cadherin cytoplasmic tail to the actin cytoskeleton and facilitate clustering into the junctional structure, forming cadherin/catenin complexes. This tail comprises two main domains: the juxtamembrane domain (JMD), which has been suggested to play a critical role in cadherins stability at the cell membrane, and the catenin-binding domain (CBD), which is known to be essential for cadherin function. The p120-catenin (p120ctn), β-catenin (βctn) and α-catenin (αctn) are the major documented interaction partners that bind to cadherin intracellular domains and allow the binding to the actin cytoskeleton of the cell (Green *et al.*, 2010) (Fig. 1).

P-cadherin upregulation was frequently observed in various malignant tumours, including breast, gastric, endometrial, colorectal and pancreatic carcinomas, and is correlated with poor survival of breast cancer patients (Hardy *et al.*, 2002, Imai *et al.*, 2008, Paredes *et al.*, 2005, Stefansson *et al.*, 2004, Taniuchi *et al.*, 2005). In contrast, significantly low levels of the P-cadherin gene expression were detected in a diverse panel of normal tissues (Imai *et al.*, 2008). Thus, disruption of P-cadherin signalling represents an intriguing opportunity for the development of novel targeted therapeutic agents in cancer.

## P-cadherin role in epithelial cell differentiation

Classical cadherins play important roles in maintaining the structural integrity of epithelial tissues and are mainly involved in cell differentiation during embryogenesis. There are several indications in the literature that point to the relationship between cell adhesion molecules and stem cell features, not only as biomarkers that help to isolate and characterise stem cells, but also as important mediators of stem cell activity, via modulation of signalling pathways (Raymond *et al.*, 2009). Regarding the classical cadherins, an important amount of data comes from the identification of P-cadherin as a marker of undifferentiated stem or progenitor cells (Kendrick *et al.*, 2008, Raymond *et al.*, 2009).

In a very recent study, it has been shown that *CDH3* is one of the genes that encode a surface protein that identify the pluripotent population of human embryonic stem cells (Kolle *et al.*, 2009). This expression is concomitant with the one of E-cadherin, which was

shown to be present even at the one cell stage of embryogenesis (Hyafil *et al.*, 1980) (Fig. 2A). In fact, mouse embryo implantation into the uterine epithelium involves both E- and P-cadherin. The most dramatic expression of P-cadherin was observed in the placenta, both in the embryonic and maternal regions, hence the classical denomination of placental-cadherin. The expression of P-cadherin in the uterus began with the appearance of the decidua, into which the extraembryonic cells expressing P-cadherin of implanted embryos invade to establish the embryo-maternal connection (Aplin *et al.*, 2009, Nose and Takeichi, 1986). Early reports specified low expression in human placenta (Shimoyama *et al.*, 1989), although P-cadherin is detectable where trophoblasts adjoin (cytotrophoblast-cytotrophoblast and cytotrophoblast–syncytiotrophoblast) in the first trimester villus, with some immunoreactivity still detectable at term (Aplin *et al.*, 2009) (Fig. 2A).

In contrast, E-cadherin was found expressed only in the embryonic region of placenta with a sharp boundary to the maternal region. These observations may suggest complementary roles of the two cadherins, such that P-cadherin is required for association of embryonic and maternal tissues during the late implantation stage, while E-cadherin is essential in preventing the embryonic tissues from mixing with the maternal tissues (Aplin *et al.*, 2009, Nose and Takeichi, 1986) (Fig. 2A).

It was also shown that E- and P-cadherins are both expressed in the ectoplacental cone, ectoderm, some endodermal tissues and nephric tubules, whereas both P- and N-cadherins are expressed in each cell of the lateral plate mesoderm, corneal endothelium, and pigmented retina (Nose and Takeichi, 1986) (Fig. 2A).

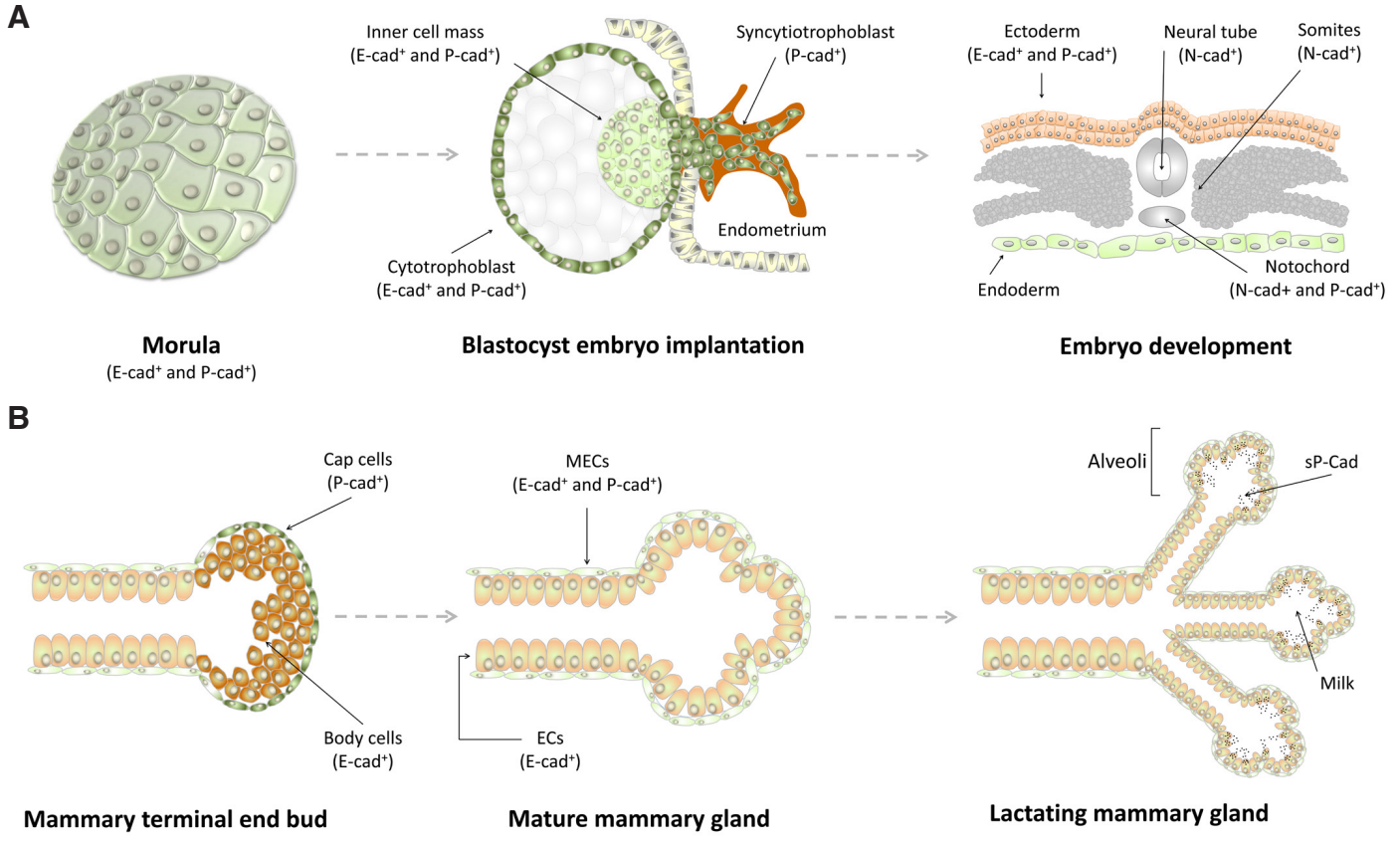


**Fig. 2. Schematic representation of P-cadherin expression during embryogenesis and mammary gland development. (A)** *Undifferentiated embryonic stem cells included in the morula, as well as in the inner mass of the blastocyst express E- and P-cadherin. During the blastocyst embryo implantation in the endometrial lining of the uterus, the trophectoderm differentiates into the cytotrophoblast and syncytiotrophoblast, which are key steps in placental development. There is an E-cadherin downregulation in the syncytiotrophoblast, which mainly expresses P-cadherin, while cells actively invade the uterine wall. Early in embryonic development, there is the formation of the neural tube, where a strip of specialized cells, forming the notochord, induces the cells of the ectoderm directly above it to become the primitive nervous system. Meanwhile, the ectoderm and endoderm continue to curve around and fuse beneath the embryo to create the body cavity, completing the transformation of the embryo from a flattened disk to a three–dimensional body. It is known that the ectoderm is E- and P-cadherin positive, which will give rise to the skin and its appendages. After cadherin switch, the neural tube becomes N-cadherin positive, as well as the somites. It was described that the notochord is N- and P-cadherin positive.* **(B)** *The ducts of the developing mammary gland are established, with their inner luminal epithelial cell layers (ECs) and outer myoepithelial cell layers (MECs), while the terminal end buds (TEB) move through the mammary fat pad. It is thought that cap cells at the tip of the TEB, which are P-cadherin positive, generate transit cells of the myoepithelial lineage on the outer side of the TEB (E- and P-cadehrin positive); at the same time, these cells also generate transit cells that form the central TEB mass, known as body cells, which will constitute the luminal epithelial lineage (E-cadherin positive). The ductal lumen is formed as body cells enter in apoptose and outer cells differentiate into luminal epithelial cells. Extracellular-matrix enzymes degrade the stroma in front of the TEB to enable it to move through the fat pad; however, it is unclear how the structures actually move through the gland. During lactation, secretory cells in the breast alveoli become P-cadherin positive at the cytoplasm, and secrete a soluble form of this protein (sP-cad) that is found in the milk.*

In adult tissues, the expression of P-cadherin is mainly found in the basal layer of several epithelial structures, such as skin, uterine cervix, prostate, and lung, contributing to the maintenance of the epithelial phenotype. The expression of cadherin molecules was extensively studied in mouse epidermis, in adulthood and during fetal development, where has been found that E-cadherin is expressed both in the basal and intermediate layers of epidermis, whereas P-cadherin is only expressed in the basal and proliferative layer (Pizarro *et al.*, 1995). Furthermore, loss of E-cadherin plays an important role in bud formation and in the acquisition of an invasive behaviour, whereas P-cadherin becomes predominant expressed later in development, namely in the growing hair follicle and in the early progenitor cells from hair germs and small hair placodes (Fujita *et al.*, 1992, Rhee *et al.*, 2006). Like hair follicles, sweat glands and mammary glands develop also from the same discrete accumulation of stem cells resting in the primitive epidermis, the outermost cell layer of an embryo, and there is strong evidence that dynamic changes in the composition of adherens junctions are important for the development of skin appendages (Fujita *et al.*, 1992).

The final evidence showing the importance of P-cadherin for the architecture and development of epithelial tissues was demonstrated by human genetic syndromes that are induced due to P-cadherin loss. *CDH3* gene mutations have been shown to cause P-cadherin functional inactivation, leading to developmental defects associated with two inherited diseases in humans: 1) hypotrichosis with juvenile macular dystrophy (HJMD) and 2) ectodermal dysplasia, ectrodactyly, and macular dystrophy (EEM syndrome). The common features of both diseases are sparse hair and macular dystrophy of the retina, while only EEM syndrome shows the additional finding of split hand/foot malformation (SHFM) (Kjaer *et al.*, 2005, Sprecher *et al.*, 2001). No defects were described for these conditions, concerning the human mammary development, or other epithelial bud structures. However, it is known that during bud patterning, a special arrangement occurs, where cells change their interaction with their neighbours and break their attachments to the extracellular matrix (ECM). Cells achieve this by activating specific transcriptional programs (Shimomura *et al.*, 2008).

## P-cadherin role in normal breast development

Two members of the cadherin family are found to be expressed in the normal adult mature non-lactating mammary gland, usually at sites of cell-to-cell contact: E-cadherin is present in both luminal epithelial (ECs) and myoepithelial cells (MECs), whereas P-cadherin is confined to the myoepithelium (Paredes *et al.*, 2002). This type of cell localization is already found during mammary gland development, since P-cadherin expression is only found in the precursor cells of the myoepithelial compartment, the cap cells of the ductal end buds, whereas luminal cells and body cells do not show any expression of P-cadherin and are typically E-cadherin positive (Daniel *et al.*, 1995) (Fig. 2B).

Besides the restricted expression of P-cadherin in the normal breast, this protein is extremely important to the establishment of the correct architecture of the tissue, as demonstrated by functional-blocking antibody experiments *in vitro* and *in vivo*. Daniel and collaborators exposed the end buds and mature mammary glands of 5 week-old virgin mice to slow-release plastic implants liberating a monoclonal antibody for P-cadherin. No effect in the luminal layer was found, but disruption of the basally located cap cell layer was clearly observed (Daniel *et al.*, 1995). Also, more recently, Chanson *et al.,* described that P-cadherin contributes specifically to the organization of the myoepithelial cell layer of the breast, since when an antibody that blocks P-cadherin function was used in an *in vitro* self-organizing assay of the human mammary bilayer, the migration of MECs, occurring during normal sorting of both layers, was compromised (Chanson *et al.*, 2011). These experiments indicate that selective expression of P-cadherin in the basal layer is necessary for the maintenance of mammary tissue integrity.

In fact, deletion of P-cadherin affects normal mammopoiesis, since the *CDH3*-null female mice exhibit precocious mammary gland differentiation in the virgin state, and breast hyperplasia and dysplasia with age (Radice *et al.*, 1997). These observations in knockout animals indicate P-cadherin cell-cell interactions and signalling as regulatory determinants of the negative growth of the luminal epithelium, being important for the maintenance of an undifferentiated state of the normal mammary gland.

Interestingly, the expression of this adhesion molecule is activated in human mammary luminal cells during late pregnancy and lactation (Soler *et al.*, 2002). However, in these alveolar lactating cells, P-cadherin expression pattern is not restricted to the cell-cell borders, but shows a cytoplasmic staining, typical of a secreted protein. Indeed, in human milk, a soluble fragment of P-cadherin (sP-cad) with 80KDa was found to be present, corresponding to the extracellular domain of the molecule (Soler *et al.*, 2002) (Fig. 2B). Recently, Mannello and collaborators showed that the highest concentration of sP-cad is detected in milk collected during the first trimester of lactation (Mannello *et al.*, 2008). Still, it is not clear which is the biological and physiological role attributed to this fragment in the normal function of the breast. Some authors suggest a role for sP-cad in alveolar differentiation during lactation, or in the immune response of the mother or the baby, or as a signalling protein between epithelial and myoepithelial cells. Further studies are in progress to determine the sites of proteolysis of the sP-cad-secreted protein in different body fluids where it has been previously described (such as milk, serum, semen, nipple aspirate fluid (NAF), breast cyst fluid and amniotic fluid) (Mannello *et al.*, 2008, Soler *et al.*, 2002).

## Prognostic relevance of P-cadherin in breast cancer

As mentioned above, P-cadherin is expressed in normal breast MECs and in MECs associated with non-invasive breast proliferations, showing no significant cross-reactivity with luminal/ECs, stromal myofibroblasts and blood vessels (Reis-Filho *et al.*, 2003). However, P-cadherin was described as being overexpressed in 20% to 40% of invasive breast carcinomas, as well as in 25% of ductal carcinomas *in situ* (DCIS) (Paredes *et al.*, 2007a, Paredes *et al.*, 2007b, Paredes *et al.*, 2002). Most important, several studies have reported P-cadherin as a marker of poor prognosis in breast cancer, since P-cadherin-positive carcinomas were significantly associated with short-term overall and disease-specific survival, as well as with distant and loco-regional relapse-free interval (Gamallo *et al.*, 2001, Paredes *et al.*, 2005, Peralta Soler *et al.*, 1999, Turashvili *et al.*, 2011).

P-cadherin expression has also been positively associated with high histological grade tumours, as well as with well-established

markers of poor prognosis, like Ki-67, epidermal growth factor receptor (EGFR), cytokeratin 5 (CK5), vimentin, p53, and HER2 expression and negatively associated with age at diagnosis, hormonal receptors (ER and PgR), and Bcl-2 expression. Interestingly, none of these reports showed a significant association with tumour size and lymph node metastasis (Gamallo *et al.*, 2001, Paredes *et al.*, 2005, Peralta Soler *et al.*, 1999, Turashvili *et al.*, 2011).

Besides the strong association between P-cadherin expression, poor patient prognosis and tumour aggressiveness, transgenic mice overexpressing *CDH3*/P-cadherin in the luminal epithelial layer of the mammary gland, under the control of the MMTV promoter, showed normal morphogenesis, architecture, lactation and involution, and no mammary tumours formed spontaneously (Radice *et al.*, 2003). Nevertheless, Mannello *et al.,* demonstrated a significant increased shedding of sP-cad in NAFs from women with breast cancer when compared with healthy subjects or with women with pre-cancer conditions, suggesting its possible release via proteolytic processing in cancer cells (Mannello *et al.*, 2008).

## P-cadherin: marker of histological and molecular subtypes in breast cancer

Besides the strong association between P-cadherin expression and poor patient prognosis, no significant correlation was ever observed between this protein and a specific breast cancer histological type. The majority of positive P-cadherin tumours are invasive ductal carcinomas NOS, or carcinomas with metaplastic or medullary features (Paredes *et al.*, 2005, Reis-Filho *et al.*, 2003, Turashvili *et al.*, 2011). The observation that metaplastic and medullary breast carcinomas are consistently immunoreactive for P-cadherin supports a myoepithelial/basal transcriptomic programme for these lesions (Han *et al.*, 1999, Jacquemier *et al.*, 2005). Han and coworkers reported P-cadherin expression in almost all studied cases of medullary, carcinosarcomas, and sarcomatoid metaplastic breast carcinomas (Han *et al.*, 1999); in addition, all the metaplastic cases that we have studied were positive for at least one basal/myoepithelial marker, including P-cadherin (Reis-Filho *et al.*, 2003). We also showed that P-cadherin expression, in canine malignant tumours, was significantly related to spindle cell carcinoma, carcinosarcoma and osteosarcoma. In these lesions, both carcinomatous and sarcomatous components of carcinosarcoma expressed P-cadherin (Gama *et al.*, 2004, Gama *et al.*, 2008).

Concerning molecular profiling classification, at least five subtypes of invasive breast carcinoma were identified (Luminal A and B, Normal-like, HER2-overexpressing and Basal-like), exhibiting distinct clinical prognostic behaviour (Perou *et al.*, 2000). P-cadherin is one of the most important biomarkers to identify basal-like and HER2-overexpressing breast cancers (Arnes *et al.*, 2005, Paredes *et al.*, 2007b, Turashvili *et al.*, 2011). Basal-like breast cancer expresses genes characteristic of basal epithelial cells, which include, besides P-cadherin, high-molecular weight basal cytokeratins (CK5/6, CK14, CK17), vimentin, αB-crystalline, caveolins1/2 and EGFR (Arnes *et al.*, 2005). Until now, the most accepted criterion to identify basal-like breast carcinomas, by immunohistochemistry, is the triple negative phenotype along with CK5 and/or EGFR positivity (Nielsen *et al.*, 2004). However, we demonstrated that P-cadherin expression shows higher sensitivity to distinguish the basal phenotype of breast carcinomas, being a reliable option compared to the "gold standard" pair CK5/EGFR (Sousa *et al.*, 2010). Although this still need validation by gene expression profiles, these results can introduce the idea of using P-cadherin as an additional option in the daily workup of breast pathology laboratories to identify basal-like breast cancers.

P-cadherin is also prominently expressed in inflammatory breast cancer (IBC), which is a distinct and aggressive form of locally-advanced breast cancer, with high metastatic potential and high death rate. These tumours are characterized by frequent basal and HER2 phenotypes but, surprisingly, luminal IBC also express the basal marker P-cadherin (Ben Hamida *et al.*, 2008). This profile suggests a specificity that needs to be further investigated.

Interestingly, the expression profiling of *BRCA1*-deficient hereditary tumours has identified a pattern of gene expression similar to basal-like breast tumours (Palacios *et al.*, 2003). Very recently, Gorski *et al.* showed that *BRCA1* and *c-Myc* form a repressor complex on the promoters of specific basal genes, including *CDH3* gene, and represent a potential mechanism to explain the observed overexpression of key basal markers in *BRCA1*-deficient tumours (Gorski *et al.*, 2010). Actually, it has been shown that P-cadherin expression in breast carcinomas is strongly associated with the presence of *BRCA1* mutations (Arnes *et al.*, 2005).

## P-cadherin role in adhesion, invasion and motility

Carcinomas progress by promotion of local invasion and distant metastasis. The acquisition of this invasive behaviour is one of the first steps in the metastatic process. Those cancer cells often develop alterations in their shape, as well as in their attachment to other cells and to ECM. Therefore, cell-cell and cell-matrix interactions play the most important role during tumour progression, since disruption of cell-cell adhesion during carcinogenesis is the basis for motility, invasion and metastasis of tumour cells (Yilmaz and Christofori, 2010).

P-cadherin has been detected as altered in various human tumours, but its effective role in the carcinogenesis process remains discussible, since it behaves differently depending on the studied tumour cell model and context. For instance, in a colorectal cancer cell line (HT-29), P-cadherin has been suggested to act as a pro-adhesive and anti-invasive/anti-migration molecule, exactly as E-cadherin (Van Marck *et al.*, 2011). Also, in melanomas, P-cadherin behaves as an invasion suppressor gene. Indeed, in highly invasive melanoma cell lines (that lack E-cadherin expression), P-cadherin overexpression was able to promote the formation of cell-cell contacts and counteract invasion (Van Marck *et al.*, 2005). The anti-invasive effect of P-cadherin was also recently confirmed in *in vivo* experiments, showing that its expression is refractory to invasive signals induced by myofibroblasts. Nevertheless, it was found a secreted truncated variant of P-cadherin in malignant melanomas, which negatively regulates cell-cell adhesion and induces a more motile phenotype, thus playing an important role in migration and metastasis of melanoma cells (Bauer and Bosserhoff, 2006).

On the other hand, in several other models, including breast cancer, P-cadherin behaves as an oncogene, and is often reported to correlate with increased tumour cell motility and invasiveness when aberrantly expressed (Cheung *et al.*, 2010, Mandeville *et al.*, 2008, Paredes *et al.*, 2007a, Paredes *et al.*, 2004, Taniuchi *et al.*, 2005, Van Marck *et al.*, 2011). Using *in vitro* breast cancer cell models, we found that overexpression of P-cadherin promotes single cell motility, directional cell migration, as well as invasion

capacity through the matrigel matrix (Ribeiro *et al.*, 2010). This same migratory phenotype was observed in bladder, pancreatic and cholangiocarcinoma cancer cell lines (Baek *et al.*, 2010, Mandeville *et al.*, 2008, Taniuchi *et al.*, 2005, Van Marck *et al.*, 2011).

Curiously, we have noticed that P-cadherin is able to induce invasion only in cell systems which already express an endogenous and functional cadherin, like E-cadherin in breast cancer cells, or N-cadherin in HEK293T cells and PDAC pancreatic cancer cells (Paredes *et al.*, 2004, Ribeiro *et al.*, 2010, Taniuchi *et al.*, 2005). Based on this hypothesis, we have recently proved that P-cadherin is able to interact with E-cadherin in breast tumours and cancer cells, promoting cancer cell invasion by disrupting the interaction between E-cadherin and both p120ctn and βctn. In the absence of E-cadherin expression, in the same cancer model, P-cadherin is able to suppress invasion by its strong interaction with catenins, surrogating the role of E-cadherin in cell-cell adhesion (unpublished data).

## P-cadherin role in EMT and cadherin switch

Among the cadherin families, E-cadherin and N-cadherin are the most highly characterized subgroup of adhesion proteins. E-cadherin is ubiquitously expressed throughout most epithelial tissues and serves as a negative regulator to functionally block the βctn signalling pathway and suppress tumour cell growth and invasion (Onder *et al.*, 2008). However, numerous preclinical and clinical studies have shown that the loss of E-cadherin occurs concurrently with the upregulation of N-cadherin or other cadherin family members implicated in invasive growth, like P-cadherin or cadherin-11. This process, known as cadherin switching, has been reported to promote epithelial-to-mesenchymal transition (EMT) and leads to tumour cell invasion and metastasis (Thiery *et al.*, 2009).

Indeed, the switch from E- to N-cadherin is the one better known and reported by several studies. N-cadherin overexpression, via cadherin switching, was observed in various invasive cancer cell lines and tumours, namely from the esophagus, prostate, cervix, and ovary. This specific cadherin switch leads to the inhibition of cell-cell contacts and elicits active signals that support tumour-cell migration, invasion, and metastatic dissemination (Thiery *et al.*, 2009).

The cadherin switch from E- to P-cadherin is a common event during embryo development; however, few reports describe it during tumour progression. Indeed, some invasive and aggressive epithelial tumours, namely the local advanced IBC, and some highly metastatic breast cancer cells, as the 4T1 cell model, maintain E-cadherin expression at the cell membrane and show aberrant concomitant expression of P-cadherin (Ben Hamida *et al.*, 2008, Lou *et al.*, 2008). Nevertheless, there are some reports showing a switch from these two epithelial cadherins during tumour progression, namely in ovarian, endometrial and bladder carcinoma (Bryan *et al.*, 2008, Patel *et al.*, 2003, Stefansson *et al.*, 2004). In all these studies, P-cadherin increased expression significantly correlated with decreased E-cadherin expression and, consequently, represented a key step in disease progression. However, it has been already shown that, in cholangiocarcinoma cells, the E- to P-cadherin switch does not induce EMT signalling, since does not affect the expression of mesenchymal markers, such as Snail 1 and 2, vimentin, and fibronectin (Baek *et al.*, 2010).

## Recognized regulators of *CDH3*/P-cadherin transcription

Signalling pathways or other cellular mechanisms that are involved in the regulation of cadherin-mediated adhesion are thought to underlie the dynamics of the adhesive interactions between cells.

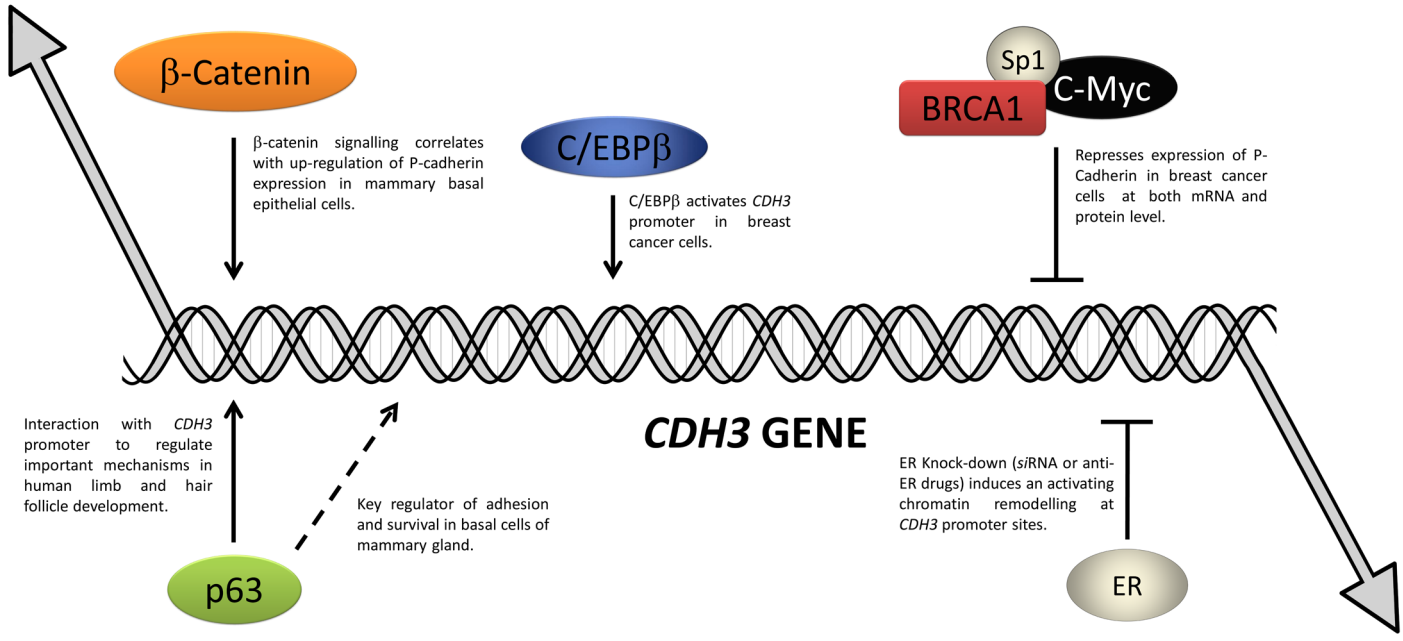


**Fig. 3. Schematic representation of the described transcriptional regulators of *CDH3* /P-cadherin promoter gene.** *It has been shown that β-catenin, p63 and C/EBPβ are transcriptional activators of* CDH3 *promoter, inducing its expression at the mRNA and protein level. In contrast, estrogen receptor (ER), as well as the BRCA1/c-Myc/Sp1 complex, act as transcriptional repressors of* CDH3 *promoter gene.*

Although the evidence that the expression of cadherins can result from growth factors and from changes in the promoter regions of cadherins, data concerning *CDH3* promoter regulation is still very limited.

One of the most prominent demonstrations regarding the importance of a classical transcription factor in the regulation of cell adhesion programmes in epithelial cells was demonstrated by Carroll and collaborators. This study implicated p63, a p53-family related transcription factor, as a key regulator of adhesion and survival in basal cells of the mammary gland. Importantly, the authors showed that p63 expression caused downregulation of cell adhesion-associated genes and detachment between mammary epithelial cells (Carroll *et al.*, 2006). This involvement of p63 in cell adhesion mechanisms was finally linked with *CDH3* gene, when Shimomura and colleagues demonstrated that P-cadherin is a direct p63 transcriptional target and that this interplay has a crucial role in human limb bud and hair follicle development (Shimomura *et al.*, 2008) (Fig. 3).

Furthermore, it has been shown that βctn is also associated with *CDH3* promoter activation and P-cadherin expression in basal mammary epithelial cells. Down-regulation of endogenous βctn levels inhibited *CDH3* promoter activity, while activation of βctn signalling was correlated with up-regulation of P-cadherin expression in *in vivo* mammary gland mice models, eventually contributing to the establishment of the basal phenotype (Faraldo *et al.*, 2007) (Fig. 3).

Recently, we still found that the CCAAT/enhancer-binding protein β (C/EBPβ) transcription factor was able to activate *CDH3* promoter in breast cancer cells. We showed that this novel activator of *CDH3* promoter activity exerts its activation preferably through its truncated LIP isoform, being the abundance of Sp1 sites within *CDH3* promoter a feature which potentiate the C/EBPβ-LIP activation role on *CDH3* gene (Albergaria *et al.*, 2010) (Fig. 3).

Regulation of *CDH3* gene has been also explored in terms of its transcriptional repression. In 2004, our group explored the link between ER-signalling and the regulation of P-cadherin expression in breast cancer cell lines, since we have already observed that breast tumours positive for P-cadherin expression were essentially ER negative. We verified that P-cadherin expression was induced by the pure anti-oestrogen ICI 182,780 and counteracted by 17β-oestradiol. In fact, breast cancer cells treated with ICI 182,780 showed a significant increase of P-cadherin mRNA and protein levels in a time and dose dependent manner, establishing that the lack of ER-signalling is responsible for the increase of P-cadherin, therefore, categorizing *CDH3* as an ER-repressed gene (Paredes *et al.*, 2004) (Fig. 3). Very recently, in order to deeply explore this antiestrogen-mediated mechanism, we described a cellular adaptation process where ICI 182,780 is able to induce a chromatin structural remodelling, which lead to activation of *CDH3* gene and overexpression of P-cadherin protein (Albergaria *et al.*, 2010). Such genomic de-repression effect may contribute to an augmented invasive phenotype of ER-positive breast cancer cells.

As a gene associated with the basal-like phenotype in breast cancer, *CDH3*/P-cadherin gene was recently described to be transcriptionally repressed by functional BRCA1 protein in breast cancer cell lines, at both mRNA and protein level. This same study also showed that, together with BRCA1, c-Myc form a repressor complex on the *CDH3* promoter (Fig. 3), suggesting a potential mechanism to explain the observed overexpression of key basal markers in BRCA1-deficient tumours (Gorski *et al.*, 2010).

## Epigenetic regulation of P-cadherin expression

Epigenetic regulation of *CDH3*/P-cadherin has been highly reported in the last few years, with greater emphasis in cancer models. The epigenetic deregulation of P-cadherin was firstly demonstrated by Sato *et al.*, which identified *CDH3* gene promoter to be aberrantly methylated in 20% of pancreatic cancers, but not in normal pancreatic epithelia (Sato *et al.*, 2003). Similarly, *CDH3* gene was also shown to be silenced by methylation in melanoma cells (Tsutsumida *et al.*, 2004).

However, in 2005, we analysed P-cadherin promoter methylation in normal breast tissue, from which only epithelial cells were microdissected, and methylation of *CDH3* gene promoter was found in the normal epithelial/luminal cell layer from all the specimens analysed, which was associated with negative P-cadherin expression in these cells. But, in contrast to what has been verified in E-cadherin control of expression by hypermethylation of its promoter in cancer, our group found a significant correlation between P-cadherin overexpression and *CDH3* promoter hypomethylation. Using a large series of invasive breast carcinomas, we found that 71% of P-cadherin-negative breast cancer cases were methylated for the *CDH3* gene, whereas 65% of P-cadherin-positive cases were unmethylated (Paredes *et al.*, 2005).

Indeed, the genomic structure of the proximal *CDH3* gene promoter, such as the enrichment in CpG islands, as well as the attributed DNA hypersensitive sites, suggests that it is likely to be regulated by epigenetic events, others than only methylation. In fact, we observed an up-regulation of *CDH3* promoter activity and P-cadherin protein expression in cells treated with the histone deacetylases (HDAC) inhibitor Trichostatin A (TSA), showing that chromatin-activating modifications are also important in the modulation of this gene (Albergaria *et al.*, 2010). Thus, if we previously described that overexpression of P-cadherin could result from a loss of promoter methylation, we have now evidences to assume that chromatin remodelling also play an important modulator role in *CDH3* gene activity.

Reinforcing our results, *CDH3* promoter was also found hypomethylated in colonic aberrant crypt foci, in colorectal cancer, and, occasionally, in the normal epithelium adjacent to cancer (Milicic *et al.*, 2008). This hypomethylation pattern was associated with the induction of P-cadherin expression in the neoplastic colon. Finally, demethylation of the *CDH3* gene was recently detected in a large percentage of primary gastric carcinomas and was significantly associated with increasing TNM stage, suggesting that it is also a frequent event in gastric carcinomas (Kim *et al.*, 2010).

## P-cadherin-downstream signalling pathways

Increasing evidences indicate that cadherins role in carcinogenesis and tumour progression do not solely lie on their adhesive function, but also depend on their interaction with other molecules (such as cytoskeletal components, integrins, and growth-factor receptors, among others) and signalling pathways (Onder *et al.*, 2008). Therefore, the stabilization of the cadherin/catenin complex represents a major regulatory mechanism for oncogenic signalling pathways, that guide cell fate decisions through the modulation of specific genes at the transcriptional level and, as a consequence, regulation of several crucial cellular processes, as proliferation, survival, polarization, differentiation, shape and migration, which

in turn affect embryogenesis, tissue formation and pathogenic events, such as cancer.

Although E-cadherin-induced signalling pathways have been extensively studied in cancer, little is known about the role of P-cadherin (Paredes *et al.*, 2004, Taniuchi *et al.*, 2005, Van Marck *et al.*, 2005). It is some kind expected that P-cadherin share common signalling pathways with other cadherins, due to its function as a cell-cell adhesion molecule; however, it is not known whether the pathways are triggered in the same way.

Sarrió and collaborators analysed microarray gene expression of a breast cancer cell line (MDA-MB-231), negative for cadherins, after expression of E- and P-cadherin. The data revealed that these molecules can activate signalling pathways leading to significant changes in gene expression. Although the expression patterns induced by E- and P-cadherin showed more similarities than differences, 40 genes were differentially modified by the expression of either cadherin type. According to data bases, these genes belonged to a wide range of biological functions, including signal transduction and growth factors (VEGFC, FGFR4), cell cycle (CCNA2), cell adhesion and ECM (CDH4, COL12A1), or cytokines and inflammation (IL24), among others (Sarrio *et al.*, 2009). This indicates that, in addition to their role in cell adhesion, E-cadherin and P-cadherin have a significant impact on the overall genetic program of breast cancer cells.

One of the molecules that have been several times referred has having a specific role in signalling related to P-cadherin is p120ctn (Fig. 4). We demonstrated that the pro-invasive activity of P-cadherin requires the JMD of its cytoplasmic tail. Transfection of HEK293T cells with several mutants of P-cadherin showed that only the ones with altered JMD were not able to induce cell invasion in *in vitro* cell models (Paredes *et al.*, 2004). Moreover, we observed that breast carcinomas co-expressing E- and P-cadherin were associated with p120ctn cytoplasmic localisation and poor patient survival (Paredes *et al.*, 2008). Since then, several other reports have been exploring that pathway. Indeed, Taniuchi *et al.,* showed that the induced cell migration by P-cadherin expression was due to activation of the Rho

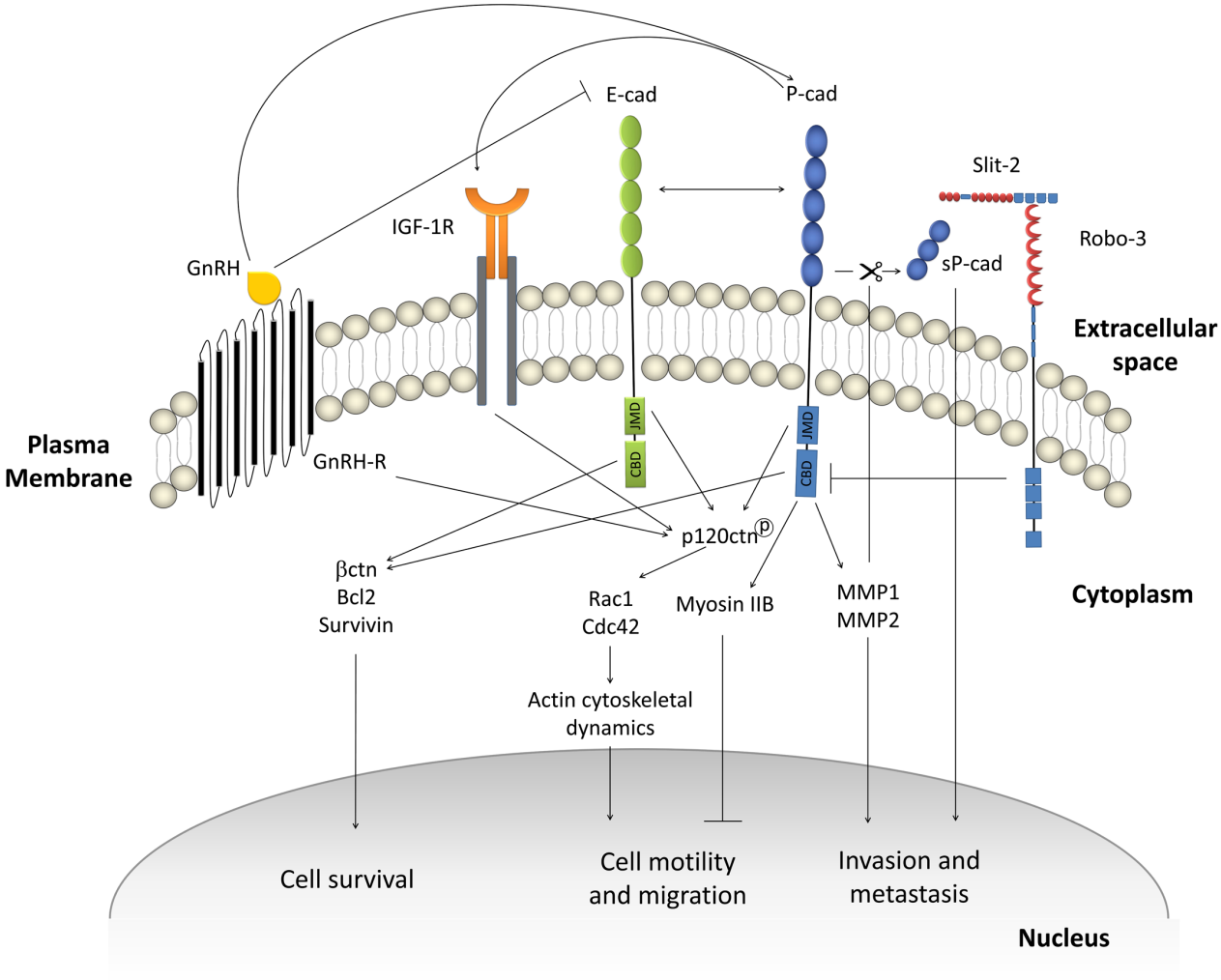


**Fig. 4. Schematic representation of the signalling pathways regulated by P-cadherin expression.** *P-cadherin signals are transduced by many intracellular signalling pathways, which ultimately result in alterations of the cancer cells survival, as well as cell migration and invasion capacity. For simplicity, only some of the known interactions are depicted. It should be noted that the effect of P-cadherin on the overall gene expression program of cancer cells is highly dependent on the cellular type and the biological context.*

GTPases, Rac1 and Cdc42, through accumulation of p120ctn in the cytoplasm in pancreatic cancer cell model (Taniuchi *et al.*, 2005) (Fig. 4). Very recently, P-cadherin has been also shown to cooperate with insulin-like growth factor-1 receptor to promote metastatic signalling of gonadotropin-releasing hormone in ovarian cancer via p120ctn (Cheung *et al.*, 2010). These same authors had previously shown that this p120ctn signalling mediated by P-cadherin expression, also lead to increased activity levels of Rac1 and Cdc42 (Fig. 4). Still another study has shown that p120ctn and P-cadherin, but not E-cadherin, regulate cell motility and invasion of DU145 prostate cancer cells (Kumper and Ridley, 2010).

Although binding of proteins to the JMD of P-cadherin has just been documented for p120ctn (Reynolds *et al.*, 1996), other molecules, like Hakai and presenilin-1 (PS-1), have been reported to bind to the JMD of classical cadherins. This binding is established through a sequence adjacent to, or overlapping, the p120ctn-binding domain, thereby competing with p120ctn (Baki *et al.*, 2001, Fujita *et al.*, 2002). Although the significance of these interactions is not well known, we cannot exclude the possibility that disruption of the p120ctn–binding sequence may introduce conformational changes and/or uncouples the interaction of these or other proteins, which could explain our observations. Striking examples of this were shown for E-cadherin, where functional differences have been noted between larger and minimal deletions of the JMD, with even the minimal changes disrupting binding of multiple molecules (Baki *et al.*, 2001).

Recently, it has been shown that the P-cadherin regulatory role in cell migration is also related with the expression of the non-muscle myosin II-B isoform, which is an ATP-dependent molecular motor protein that can interact with and contract filamentous actin (F-actin) (Jacobs *et al.*, 2010) (Fig. 4). These results implicate that there is a coordinated cross-talk between adhesion molecules and cellular migration-related proteins.

More recently, the role of P-cadherin was investigated in oral squamous cancer cell model, where the authors used a cell line that was deficient for classical cadherins. After P-cadherin overexpression, cells gained an epithelial-like morphology, with Snail translocation to the cytoplasm. Analysing the signalling mechanism behind it, they found glycogen-synthase-kinase-3β (GSK-3β) bound to Snail, as well as an increase in activated GSK-3β that phosphorylated Snail leading to its cytoplasmic translocation (Bauer *et al.*, 2009). These same authors also showed that Slit-2, a secreted ECM glycoprotein that acts as a molecular guidance cue in cellular migration, facilitates the interaction of P-cadherin with Robo-3, its receptor, and inhibits cell migration in oral squamous cell carcinoma cell line models (Bauer *et al.*, 2011) (Fig. 4).

In terms of breast cancer cell invasion, we found that the presence of P-cadherin, in an E-cadherin positive cellular background, is able to provoke the secretion of pro-invasive factors, such as MMP-1 and MMP-2, leading to P-cadherin ectodomain cleavage (sP-cad) which induces a pro-invasive activity by itself (Ribeiro *et al.*, 2010). This study clarified the mechanism associated to P-cadherin-induced cancer cell invasion.

Different signalling pathways should be triggered in different cell models, in order to identify new interaction partners of P-cadherin, as well as to study whether the interaction of known partner molecules differ between cadherins. Finally, it is important to highlight that the effect of cadherins on the overall gene expression program of cancer cells is highly dependent on the cellular type and the biological context. Thus, P-cadherin regulation of specific transcriptional factors may depend on the activation of other signalling pathways, or on the presence of additional molecular alterations.

## P-cadherin as a breast cancer stem cell marker

An increasing body of evidence supports the notion that cancers are propagated by a small population of cells present in the malignant tissue, that possess the ability to form a hierarchy similar to the one present in normal tissues (Visvader, 2011). These cancer stem cells (CSCs) are able to proliferate, originating more stem-like cells, to exhibit resistance to current therapies and to remain quiescent during long periods of time. However, it is still not clear whether the CSC originates from the normal stem cells of the tissue that deregulate their self-renewal ability, or from normal mature cells or progenitor cells that acquired stem cell characteristics (Visvader, 2011). Importantly, attempts have been made in order to find a universal phenotype for the breast cancer stem cell; but due to the high heterogeneity of this malignancy, it is not expected that a single CSC phenotype would apply to all breast cancers.

The identification of a cancer stem cell marker for basal-like subtype of breast cancer is of particular importance, due to its high mortality rate, fast relapses and lack of target therapy (Rakha *et al.*, 2009). Recently, it has been demonstrated that the luminal progenitor of normal breast hierarchy is the cell of origin for this malignancy, since the induction of a *BRCA1* mutation in this cell was able to induce the formation of a tumour with basal phenotype (Lim *et al.*, 2009, Molyneux *et al.*, 2010). Since *CDH3* gene is repressed by BRCA1, it is likely that P-cadherin could be a good cancer stem cell marker of this specific type of tumours. In fact, using a series of breast cancer cell lines, we found that P-cadherin enriched populations (by genetic manipulation or by sorting) were enriched for mammosphere forming efficiency (MFE), as well as for the expression of CD24, CD44 and CD49f, already described as CSC markers. When compared with luminal cell lines, basal-like cell lines also showed a greater ALDEFLUOR$^{bright}$ subpopulation and the P-cadherin positive subfraction of these cell lines was enriched in stem cell activity (MFE and 3D growth) (unpublished data). This observation linked P-cadherin expression with the luminal progenitor phenotype, which is CD44$^+$CD24$^+$CD49f$^+$ (Lim *et al.*, 2009). Importantly, it has been described that the phenotype CD44$^+$CD24$^+$ is tumorigenic (Meyer *et al.*, 2009). Hence, the strategy of directing therapies to the luminal progenitor phenotype, by specifically targeting P-cadherin, could potentially help to eradicate the CSCs. Interestingly, P-cadherin also conferred resistance to X-ray induced DNA damage, supporting a role for this molecule in the maintenance of yet another CSC property (unpublished data).

## P-cadherin - potential therapeutic target in cancer

As clearly stated in this review, P-cadherin–mediated adhesion and the associated signalling pathways play diverse roles in the regulation of cancer cell survival, invasiveness and metastatic potential. Interestingly, in 2008, Imai and collaborators have suggested *CDH3*/P-cadherin as a possible target for immunotherapy of pancreatic, gastric, and colorectal cancers, since it was identified as a novel tumour-associated antigen, meaning that was strongly expressed in tumour cells, but not in normal cells (Imai *et al.*, 2008). Indeed, we have found that P-cadherin silencing, in breast cancer

cells inoculated in nude mice, was able to significantly inhibit *in vivo* tumour growth (unpublished data).

Recently, a novel and highly selective human monoclonal antibody against P-cadherin (PF-03732010) was produced, demonstrating anti-tumour and anti-metastatic activity in a diverse panel of P-cadherin–overexpressing tumour models, without introducing any adverse secondary effects in mice (Zhang *et al.*, 2010). This antibody failed to bind to the most closely target-related family members, including E-cadherin, N-cadherin, and VE-cadherin. PF-03732010 also reduced lymph node metastases and lowered the levels of circulating tumour cells (CTC) in whole blood of P-cadherin$^+$ tumour bearing mice. The anti-metastatic property of the antibody was remarkable, since it significantly inhibited tumour cell infiltration into the lungs. PF-03732010 still suppressed βctn, cyclin D1, Vimentin, Bcl-2, and survivin expression, decreased the Ki67 levels, and increased caspase-3 expression (Zhang *et al.*, 2010) (Fig. 4).

Taken together, these recent data highlight the critical role of P-cadherin signalling in regulating tumorigenesis and metastasis, especially because its inhibition leads to anti-tumour and anti-metastatic effects in target-associated tumour models without any adverse indication. These observations provide the rationale and guidance for the clinical development of PF-03732010, in which tumours with high P-cadherin expression will be essential criteria for patient selection. Future work is warranted to seek a reproducible method to quantify P-cadherin in human tumours and to find a reasonable cut-off of expression related with therapeutic response, in an attempt to reach the full potential for clinical development of the antibody. PF-03732010 is currently under Phase I clinical trial development.

## Conclusions

Although this review is mainly focused on P-cadherin role as a poor prognostic factor, as well as a therapeutic target in breast cancer, its upregulation is also found in several other malignancies, affecting organs such as pancreas, stomach, bladder and prostate, where it is also associated with an aggressive phenotype and poor prognosis. Thus, antagonizing P-cadherin represents a novel approach for anticancer therapy, by targeting tumours with high P-cadherin expression. Interestingly, P-cadherin silencing induces significant growth inhibition in several tumour models tested; however, this anti-proliferative activity was never observed *in vitro* (Zhang *et al.*, 2010). This discrepancy suggests that fully functioning P-cadherin signalling may require the cell-cell and cell-stroma crosstalk in intact tumour architecture during tumorigenesis and metastasis, a process that may not be recapitulated under *in vitro* conditions and that should be further studied in the future.

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