

Chapter 7

Cell Polarity: A Key Defence Mechanism Against Infection and Cancer Cell Invasion?

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Abstract It is now emerging that a number of cellular targets of pathogens are involved in the establishment and/or maintenance of epithelial cell polarity. Increasing evidence also suggests that cancer-causing pathogens such as *Helicobacter pylori* (*H. pylori*) and human papilloma virus (HPV) may induce oncogenesis by disrupting cell polarity. This is mainly achieved through their ability to deregulate the function of cell polarity components and/or regulators. Hence cell polarity represents the first line of defence against infection. Interestingly, EGFR/RAS oncogenic signals also induce cancer cell invasion by inducing epithelial to mesenchymal transition (EMT). Since the loss of cell polarity is a prerequisite of EMT, cell polarity also represents the last line of defence against cancer cell invasion. As such we argue that cell polarity may be a key defence mechanism against infection and cancer cell invasion. The potential role of cell polarity as a gatekeeper against cancer through its ability to regulate asymmetric cell division and tumour suppression has been discussed in a number of recent reviews. In this review we will focus on the role of cell polarity as a potential target of infection and cancer cell invasion.

Keywords Cancer • Bacteria *H. pylori* • Cell polarity • Cancer-causing virus HPV • Infection • RAS oncogene

7.1 Introduction

There are over 260 cell types in our body, and around 200 different types of cancer have been reported that are derived from approximately 60 different organs. To a large extent, cancers derived from different organs and cell types have distinct features and distinct genetic mutation signatures. This heterogeneous nature contributes to the complexity and difficulty in treating cancers. However, one common feature of cancer is excessive cell growth due to enhanced cell proliferation and/or

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reduced cell death. Additionally, over 80 % of human cancers originate from epithelial cells. Therefore, it is critical to study the common features of epithelial cancers as it will help us to understand why most cancers derive from epithelial cells and will also guide us to develop more effective treatment for epithelial cancers.

Epithelial cells are widely distributed, lining the surface of the animal body and internal cavities (e.g. digestive tract and circulatory system) and form many glands. They are involved in diverse functions including secretion, absorption and barrier functions. There are three main epithelial cell types: 1) squamous epithelial cells that are mainly found in the skin, oral cavity and oesophagus; 2) cuboidal epithelial cells that are located in ductal tissues such as the mammary gland and the prostate; and 3) columnar epithelial cells that are mainly located in the stomach and intestine. The common features of epithelial cells are their adherence to each other via tight junctions, desmosomes and adherence junctions, and their polarity. Epithelial cells have planar cell polarity and apical–basal polarity, and they both play crucial roles in the development and maintenance of epithelial tissue homeostasis. Apical–basal polarity is most prominent in cuboidal and columnar epithelial cells, and it is the focus of this review. It is defined by the apical membrane facing the outside surface of the body, or the lumen of internal cavities, and the basolateral membrane oriented away from the lumen. The basolateral membrane acts as a scaffold for the epithelial cells to join the underlying connective tissue and cell–cell junctions. Tight junctions (TJs) are located at the apical side of the adherens junction (AJs) and are often partially localised with the apical polarity complex. Gap junctions, desmosomes and hemi-desmosomes are located at basolateral membranes of epithelial cells and regulate communication between cells and with the extracellular matrix (ECM). TJs are crucial for epithelial barrier function providing a tight seal between the membranes of the neighbouring cells, while AJs use the forces that are generated by the actin cytoskeleton to keep the cellular membranes of neighbouring cells together (Suzuki and Ohno 2006). TJs and AJs also limit the paracellular permeability of fluid and ions between the lumen and the interstitium (Hartsock and Nelson 2008).

Three groups of proteins play a central role in the establishment and maintenance of apical–basal cell polarity: the Crumbs–PALS1 (Stardust)–PATJ (Crumbs complex) and Par3 (Bazooka)–Par6–aPKC (Par complex) complexes, which are found apically, and the lethal giant larvae (Lgl)–Scribble (Scrib)–discs large (Dlg) proteins (Scribble complex) which localise at the basolateral membrane (Margolis and Borg 2005; Suzuki and Ohno 2006). Both the Par and Crumbs complexes promote apical membrane identity, whereas the Scribble complex promotes basolateral membrane identity by antagonising the other two (Bilder 2004).

Excessive cell growth and the loss of cell polarity have long been used by pathologists as hallmarks for cancer diagnosis (Hanahan and Weinberg 2011). Extensive studies carried out by human cancer genome projects in the past 10 years have clearly illustrated that regulators of the cell cycle are frequently mutated in human cancer, emphasising the importance of cell cycle regulation in tumourigenesis. The mutation rate of genes that encode the key components of cell

polarity complexes is relatively low in human cancers, in contrast with genes involved in cell cycle regulation. Instead, the genes involved in cell–cell adhesion represent an emerging class of mutated genes found in human epithelial cancers (Royer and Lu 2011; Berx and van Roy 2009). Unanswered questions still remain. Are these passenger or driver mutations? Is the observed loss of cell polarity a consequence of excessive cell growth or is it an evidence of cell polarity's tumour-suppressive function? Additionally, RAS and p53 remain to be one of the most frequently mutated oncogene and tumour suppressor pathways found in most human cancers. Therefore an intriguing question is whether the cell polarity machinery (i.e. the regulators and components of cell polarity complexes) could be regulated by oncogene and tumour suppressor pathways, in particular the RAS and p53 pathways? Epidermal growth factor receptor (EGFR)/RAS is arguably the best characterised oncogenic signalling pathway that disrupts cell polarity and cell–cell adhesion, and it induces epithelial to mesenchymal transition (EMT) and cancer cell invasion. Consistent with this, tensin homolog (PTEN), which negatively regulates the phosphatidylinositol 3-kinase (PI3K) pathway by dephosphorylating the PI3K product, phosphatidylinositol (3,4,5)-trisphosphate [PtdIns(3,4,5)P₃], and producing phosphatidylinositol (4,5)-bisphosphate [PtdIns(4,5)P₂], is a tumour suppressor that regulates cell polarity. During polarisation of epithelial cells, PTEN is targeted to the future apical membrane domain, where it generates PIP₂, which facilitates recruitment of annexin 2 (Anx2), Cdc42 and the apical Par6–Par3–aPKC complex (Martin-Belmonte et al. 2007). As apical accumulation of PIP₂ is dependent on apical targeting of PTEN and as membrane targeting of Par3 is mediated by direct binding to phosphoinositide lipids, PTEN may be instrumental in the apical localisation of Par3 (Feng et al. 2008; Dow et al. 2008; Wu et al. 2007; Martin-Belmonte et al. 2007). Recent studies have also illustrated that p53 mutation often occurs in metastatic cancers and that mutant p53 facilitates EMT and cancer cell invasion (Zhang et al. 2011). Therefore, cell polarity may play an active role during tumour genesis.

Intriguingly, apoptosis-stimulating protein of p53-2 (ASPP2), a known tumour suppressor and an activator of p53 (Samuels-Lev et al. 2001), has recently been identified as a binding partner of Par3, a regulator of apical polarity and cell–cell adhesion *in vitro* and *in vivo* (Sottocornola et al. 2010; Cong et al. 2010). ASPP1 and iASPP together with ASPP2 make up the ASPP family of proteins. ASPP1 and ASPP2 stimulate, whereas iASPP inhibits, p53's apoptotic function (Trigiante and Lu 2006). They are also characterised as ankyrin repeats, an SH3 domain and a proline-rich-region-containing proteins (ASPP). A number of transgenic mouse studies have established ASPP2 as a haploinsufficient tumour suppressor (Kampa et al. 2009; Vives et al. 2006; Tordella et al. 2013). It also acts an activator of the RAS oncogene, and the identified ASPP2–RAS interaction mediates the tumour-suppressive function of RAS oncogene including the induction of cellular senescence in primary cells and apoptosis in cancer cells (Wang et al. 2012, 2013a, b). RAS activation also induces ASPP2 translocation from cell–cell junctions to the cytosol and nucleus (Wang et al. 2013a). Importantly, ASPP2 has been identified as a cellular target of the cancer-causing pathogens *Helicobacter pylori* (*H. pylori*)

(Buti et al. 2011) and human hepatitis C virus (HCV) (Cao et al. 2004). These new findings suggest that regulators of cell polarity, such as ASPP2, may connect cell polarity to oncogenes and tumour suppressor pathways as well as to infection and tumourigenesis.

A number of cellular targets of cancer-causing pathogens are involved in the establishment and/or maintenance of epithelial cell polarity. Increasing evidence also suggests that non-cancer-causing viruses (such as influenza, dengue, tick-borne encephalitic viruses, rabies, severe acute respiratory syndrome (SARS) coronavirus and human immunodeficiency virus (HIV)) target components of cell polarity or TJs/AJs to enable efficient replication and spread of pathogens (Mothes et al. 2010; Javier and Rice 2011; Bonazzi and Cossart 2011). Hence, cell polarity represents the first line of defence against infections. Importantly, cancer-causing pathogens such as *H. pylori*, human papilloma virus (HPV), human hepatitis B virus (HBV), HCV (Moudgil et al. 2013; Thomas 2013) and human T-cell lymphoma virus (HTLV) may induce tumourigenesis by disrupting cell polarity (Fig. 7.1). This is mainly achieved via their ability to deregulate the function of cell polarity components and/or regulators. Therefore, cell polarity could be a first line of defence against cancer-causing infections and may represent a prime target of cancer-causing pathogens (Javier and Rice 2011). Interestingly, one of the most frequently mutated oncogenic signalling pathways, EGFR/RAS/PI3K, induces cancer cell invasion by inducing EMT. The loss of cell polarity is a prerequisite of EMT (Thiery 2003; Thiery et al. 2009) and an initial step of epithelial cancer metastasis. Many cancer-causing pathogens such as *H. pylori* also induce EMT upon infection. Hence cell polarity may represent the last line of defence against cancer cell invasion. By reviewing the recent studies in this area of research, we would like to present a hypothesis that cell polarity may be a key defence mechanism against infection and cancer cell invasion.

7.2 Cell Polarity: The First Line of Defence Against Infections

A unique feature of epithelial cells is their ability to form a barrier. External barriers protect organisms from water leakage. Both external and internal barriers also prevent unwanted substances from entering the skin or other organs and therefore causing cell and tissue damage. Perhaps one of the most important functions of the barrier is its ability to protect us from infections. The epithelial barrier is achieved through the ability of epithelial cells to adhere to each other through the formation of TJs sealing the epithelial sheet in a highly polarised manner. Correct orientation of highly polarised epithelial cells is also crucial for secretion and absorption. Hence, the integrity of cell polarity and cell–cell adhesion is vital in the establishment and maintenance of epithelial tissue homeostasis and organogenesis. It is therefore not surprising that cellular targets of pathogens are often involved in the

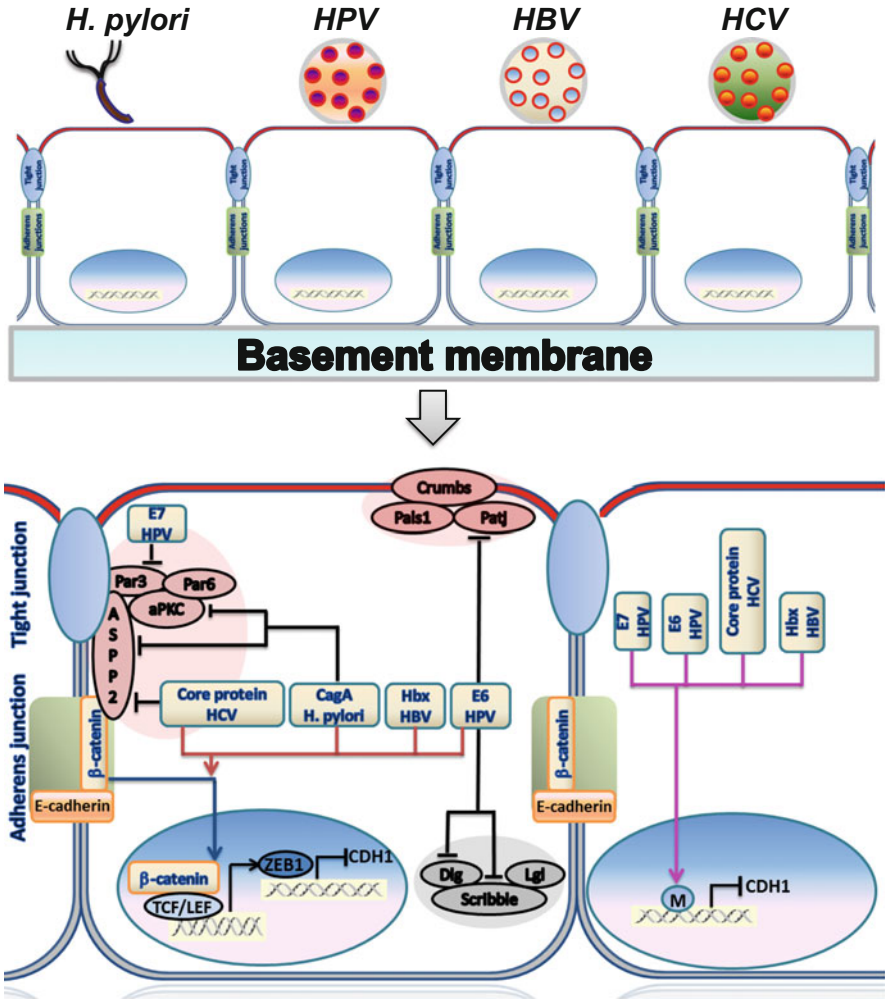


Fig. 7.1 Cell polarity is the first line of defence against infections. Epithelial cells form a barrier, which protects us from infections. Human papilloma virus (HPV) E6 and E7 proteins promote degradation of Scribble, discs large homolog (Dlg), PALS1-associated tight-junction protein (PATJ) and Par3. Apoptosis-stimulating of p53 protein 2 (ASPP2) is inhibited by *Helicobacter pylori* (*H. pylori*) CagA protein and human hepatitis C virus (HCV) core protein. *H. pylori* CagA also inhibits the function of atypical protein kinase C (aPKC), which causes junctional and polarity defects. These pathogens induce epithelial mesenchymal transition (EMT) by controlling the expression levels of E-cadherin either directly or indirectly via β-catenin, a binding partner of E-cadherin at adherens junctions and also an activator of the Wnt signalling pathway. Nuclear β-catenin complexes with T-cell and lymphoid enhancer (TCF/LEF) to transactivate the expression of ZEB1, which represses the expression of E-cadherin. Human hepatitis B virus (HBV), HBV protein HPV (E6/E7) and HCV (core protein) downregulate E-cadherin expression directly, usually via inducing methylation (M) of the E-cadherin promoter (CDH1). *H. pylori*, HPV, HBV and HCV also enhance the nuclear accumulation of β-catenin via various mechanisms. *Hbx* HBV X protein. Arrows (➔) indicate activation and T-junctions (⊥) indicate inhibition

establishment or maintenance of cell polarity and cell–cell adhesions. In particular, PDZ domain-containing proteins (PSD95/Dlg/ZO-1) (Kennedy 1995) play essential roles in most aspect of cellular homeostasis including control of cell–cell adhesion, cell polarity and cell migration. These proteins have recently been identified as cellular targets of pathogenic viruses (Javier and Rice 2011). The PDZ domain is a common structural domain of 80–90 amino acids found in the signalling proteins of bacteria, yeast, plants, viruses and animals (Ponting et al. 1997). For example, PDZ-containing basal–lateral polarity complex Dlg1 is a target of adenovirus, influenza, HPV, HTLV, HIV and rabies (Javier and Rice 2011). Additionally, cancer-causing pathogens such as the gram-negative bacteria *H. pylori*, DNA tumour virus such as adenovirus, HPV, HBV and HCV, as well as the tumour-causing retrovirus HTLV are all able to bind and deregulate the function of regulators or components of cell polarity in order to achieve their oncogenic properties (Javier and Rice 2011; Javier 2008; Banks et al. 2012). All these suggest that cell polarity represents a first line of defence against infection and is a prime target of cancer-causing pathogens (Fig. 7.1).

7.2.1 *Par Complex and ASPP2 Are Targeted by Cancer-Causing H. pylori, Papilloma Virus and Hepatitis Viruses*

H. pylori is a bacterial species that specifically colonises gastric epithelium and is associated with peptic ulcer disease, gastric adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma. *H. pylori* strains belong to two types. Type I strains contain in their genome the cytotoxin-associated gene pathogenicity island (Cag PAI) and express CagA protein, whereas Type II strains are CagA negative. *H. pylori* CagA-positive strains are associated with gastritis, ulcerations and gastric adenocarcinoma (Peek and Blaser 2002). CagA is delivered into gastric epithelial cells (Segal et al. 1999) and is able to induce the formation of an elongated cell shape (Bagnoli et al. 2005). In polarised epithelial cells, CagA causes loss of apical–basolateral polarity (Amieva et al. 2003; Bagnoli et al. 2005). *H. pylori* CagA specifically interacts with Par1 kinase, which has an essential role in epithelial cell polarity. Association of CagA inhibits Par1 kinase activity and prevents atypical protein kinase C (aPKC)-mediated Par1 phosphorylation, which dissociates Par1 from the membrane, collectively causing junctional and polarity defects (Saadat et al. 2007). Recently, it has been demonstrated that CagA interacts with ASPP2 (Buti et al. 2011; Nešić et al. 2014), a haploinsufficient tumour suppressor (Kampa et al. 2009; Vives et al. 2006) and a binding partner and a regulator of Par3 (Cong et al. 2010; Sottocornola et al. 2010). In mammalian cells, ASPP2 associates with Par3, a complex crucial for the formation and localization of the apical–junctional complex (AJC). ASPP2-depleted cells are defective in the formation of TJs and acquire a migratory phenotype (Sottocornola et al. 2010; Cong et al. 2010).

Interestingly, the CagA–ASPP2 interaction facilitates the formation of the CagA–ASPP2–p53 complex and consequently results in proteasomal degradation of p53. How this CagA–ASPP2 interaction leads to enhanced p53 degradation is currently unknown. Nonetheless the identification of ASPP2 as a prime cellular binding partner of CagA in addition to Par1 extended the list of cell polarity components as cellular targets of CagA.

It has been well established that ASPP2 functions as a tumour suppressor by enhancing the transcriptional activity of p53 (Samuels-Lev et al. 2001). Additionally, ASPP2 acts as a regulator of cell polarity and cell adhesion in a p53-independent manner. This property of ASPP2 is evolutionarily conserved. *Drosophila* ASPP (dASPP) localises at AJs and regulates the activity of C-terminal kinase (dCsk). Loss of function of dASPP increases cell spreading and apoptosis (Langton et al. 2007, 2009). The fact that ASPP2 may suppress tumour growth via both p53-dependent and p53-independent pathways places it as an ideal cellular target of oncoproteins derived from cancer-causing pathogens, such as CagA from *H. pylori*. Consistent with this, ASPP2 was also identified as a cellular target of the core protein of HCV. Hepatocytes are highly polarised and have basolateral and apical poles, separated by TJs in contact with blood vessels and bile ducts respectively (Perrault and Pecheur 2009). Chronic infections with HBV and HCV are associated with 80 % of hepatocellular carcinomas (HCCs) worldwide. Both HBV and HCV express proteins that have transforming potential and which directly affect cell polarity. This binding of HCV core protein to ASPP2 blocks the interaction between p53 and ASPP2 and inhibits p53-mediated apoptosis (Cao et al. 2004). HCV core protein is also known to be involved in disrupting cell polarity and cell–cell adhesion upon infection (Awad et al. 2013). Thus it is tempting to speculate that HCV core protein may disrupt cell polarity and cell–cell adhesion through its ability to interact with ASPP2.

It was shown recently that loss of the Par3 promotes breast tumourigenesis and metastasis (McCaffrey et al. 2012). ASPP2 is known to function as a tumour suppressor by enhancing the transcriptional activity of p53. ASPP2 and Par3 also form a protein complex that regulates cell polarity independently of p53. The junctional localisation of ASPP2 and Par3 is interdependent, and the interaction between ASPP2 and Par3 controls the integrity of cell polarity and cell–cell adhesion in vitro and in vivo (Sottocornola et al. 2010; Cong et al. 2010). Thus targeting either ASPP2 or Par3 could inactivate the ASPP2–Par3 complex and disrupt cell polarity and/or cell–cell adhesion. Like many other components of cell polarity complexes, Par3 contains a PDZ domain. Interestingly, Par3 is a target of E7 oncoprotein of rhesus papilloma virus (RhPV1), a virus closely related to HPV16, and it causes cervical cancer in the rhesus macaque (Tomaic et al. 2009). The PDZ-binding motif (PBM) of E7 interacts with Par3 and targets it for proteasome-mediated degradation (Tomaic et al. 2009). Thus, RhPV1 may cause cervical cancer in the rhesus macaque by deregulating both p53-dependent and p53-independent tumour-suppressive functions of ASPP2. p53 degradation mediated by HPV protein, E6, compromises the apoptotic function of the ASPP2–p53 complex. By targeting Par3 for proteasome-mediated degradation, E7 disrupts the

ASPP2–Par3 complex. Thus, oncogenic papilloma viruses could cause an increased resistance to apoptotic stimuli on one hand and induce EMT on the other, to impose the aggressive and invasive phenotype.

7.2.2 Crumbs and Scribble Complexes Are Targeted by Cancer-Causing Viruses

HPV is the most studied cancer-causing virus and causes a diverse range of epithelial lesions that are the causative agents of a number of human cancers, the most prominent being cervical cancer. Cervical cancer occurs following persistent infection with a high-risk viral subtype (HPV16 or 18) and is characterised by continued expression of the viral oncoproteins E6 and E7. The E6 and E7 proteins derived from high-risk HPV are able to bind and inactivate tumour-suppressive functions of p53 and Rb, respectively, to drive cell proliferation. E6 binds p53 and targets it for E6AP-mediated proteasomal degradation (Talis et al. 1998), whereas E7 binds Rb and prevents it from inhibiting the transcriptional activity of E2F and cell cycle entry (Moody and Laimins 2010). It is now emerging that E6 and E7 exert a co-ordinated attack on PDZ domain-containing proteins that are components or regulators of Crumb or Scribble cell polarity complexes.

Analysis of the sequences of E6 proteins derived from the cancer-causing mucosal HPV types reveals a remarkable conservation of amino acid sequences at the extreme carboxyl termini of the proteins. All of these E6 proteins have a class I PDZ (PSD95/Dlg/ZO-1)-binding motif (x-T/S-x-L/V) (PBM) at their carboxyl termini (Songyang et al. 1997). E6 oncoprotein has been shown to interact, through this motif, with a large number of PDZ domain-containing cellular targets (Banks et al. 2012; Pim et al. 2012; Thomas et al. 2008). The PBM of HPV E6 is able to interact with discs large (Dlg) and Scribble from the Scribble complex and PALS1-associated tight-junction protein (PATJ) of the Crumb complex. Crumb is an apical polarity complex whereas Scribble is a basolateral polarity complex (Kiyono et al. 1997; Lee et al. 1997; Nakagawa and Huibregtse 2000; Gardiol et al. 1999; Thomas et al. 2005; Storrs and Silverstein 2007). In each case, E6, by recruiting cellular ubiquitin-protein ligases, can target distinct pools of these proteins for proteasome-mediated degradation (Gardiol et al. 1999; Nakagawa and Huibregtse 2000; Storrs and Silverstein 2007; Tomaic et al. 2009; Watson et al. 2003). In many of the model systems that have been analysed, the capacity of E6 from the high-risk HPV types to retain PDZ-binding capacity has been shown to contribute towards its transforming activity, both within tissue culture model systems (Kiyono et al. 1997; Watson et al. 2003) and also in transgenic mouse models (Nguyen et al. 2003).

Like HPV, human adenovirus infects epithelial cells. The oncoprotein E4-ORF from human adenovirus targets both the Crumb and Scribble complexes. This is achieved by binding to the PDZ domains of Dlg1 and PATJ. Additionally, HTLV infects T cells that do not have an apical cell polarity complex. TAX oncoprotein

from HTLV only targets the Scribble complex as it binds the PDZ domain of Dlg1, Dlg4 and Scribble. All these argue for the importance of cell polarity in virus-induced malignancy.

7.2.3 Cancer-Causing Pathogens such as H. pylori, Papilloma Virus and Hepatitis Viruses Can Induce EMT

For a primary epithelial cancer cell to metastasise, it must first disrupt cell–cell adhesion to allow it to detach from the primary cancer site and to migrate to a new site. Disruption of cell–cell adhesion is often associated with EMT. Epithelial cells adhere to each other via TJs and AJs at the apical side and via gap junctions and desmosomes at the basolateral side. ZO1, ZO2, occludin and claudin are the main components of TJs, whereas E-cadherin and β -catenin are main components of AJs (Dejana 2004). Importantly, TJs and AJs often overlap with the apical polarity complex Par3–Par6–aPKC. Par3 and ASPP2 control the initial formation of TJs and AJs in vitro (Cong et al. 2010; Sottocornola et al. 2010). Reduced E-cadherin expression, loss of cell polarity and disruption of cell–cell adhesions are a prerequisite of EMT.

Interestingly, the PDZ-containing protein ZO2 is a cellular target of human adenovirus E4-ORF1 (Glaunsinger et al. 2001). HCV also targets TJ proteins claudin-1 and occludin to allow its cellular entry (Perrault and Pecheur 2009; Mee et al. 2008; Benedicto et al. 2009; Meertens et al. 2008; Evans et al. 2007). The actual roles of claudin-1 and occludin in HCV cell entry remain unclear, but interestingly a direct interaction between the HCV envelope glycoproteins and occludin has been shown (Benedicto et al. 2008). Furthermore, knockdown of occludin in a cell–cell fusion assay, where fusion activity depends on cell surface expression of the HCV glycoprotein complex, gave rise to diminished fusion activity, suggesting that occludin may be implied in the HCV fusion process (Benedicto et al. 2009). During infection, HCV disrupts TJs and cell polarity in various in vitro systems (Mee et al. 2010; Benedicto et al. 2008; Wilson et al. 2012). It was shown that HCV infection promotes vascular endothelial growth factor (VEGF) expression that depolarises hepatoma cells, promoting viral transmission and lymphocyte migration into the parenchyma that may promote hepatocyte injury (Mee et al. 2010). HCV glycoproteins also perturb TJ and AJ protein expression and increase hepatoma migration. This is achieved by stabilising hypoxia-inducible factor 1 α (HIF-1 α), which upregulates the expression of EMT-inducing transcription factors such as Snail and Twist (Wilson et al. 2012).

Perhaps the best characterised mechanism that many cancer-causing pathogens use to induce EMT is through their ability to control the expression levels of E-cadherin either directly or indirectly via β -catenin. Both HPV E6 and E7 are able to downregulate E-cadherin expression (D'Costa et al. 2012; Caberg

et al. 2008); the E-cadherin promoter is repressed in cells expressing E6, resulting in fewer E-cadherin transcripts. Mechanistically this is not caused by either an increase in histone deacetylase activity or a binding of trans-repressors to the E-cadherin promoter Epal element. In contrast, E6 expression induces DNA methyltransferase (DNMT) activity (D'Costa et al. 2012). It was also shown that HPV16 E7 silencing may induce E-cadherin re-expression via AP-2 transcription factor (Caberg et al. 2008). The HBV X protein (Hbx) is able to induce methylation of E-cadherin promoter to reduce expression (Lee et al. 2005). Similarly the core protein of HCV also induces E-cadherin promoter methylation to downregulate E-cadherin expression (Arora et al. 2008).

Most cancer-causing pathogens control E-cadherin expression by regulating the expression levels and cellular localisation of β -catenin, a binding partner of E-cadherin at AJs and activator of the Wnt signalling pathway (Fig. 7.1). Nuclear β -catenin forms complexes with the T-cell and lymphoid enhancer (TCF/LEF) and transactivates the expression of ZEB1 (Sanchez-Tillo et al. 2011). In turn, ZEB1 represses the expression of E-cadherin (Peinado et al. 2007). HPV16 E6 oncoprotein enhances the nuclear accumulation of β -catenin, and this effect requires an intact E6 PDZ-binding domain (Bonilla-Delgado et al. 2012). As a result, increased nuclear β -catenin represses the expression of E-cadherin (Bonilla-Delgado et al. 2012). Hbx also increases the expression levels of β -catenin by perturbing the interaction between β -catenin and the tumour suppressor, adenomatous polyposis coli (APC) (Hsieh et al. 2011). Hbx competitively binds APC to displace β -catenin from its degradation complex. This results in upregulation of nuclear β -catenin and activation of Wnt signalling (Hsieh et al. 2011). However the mechanisms with respect to the role of Wnt-5a in HBV-associated hepatocellular carcinoma (HCC) need further investigation. In addition, mutations in the C-terminus of Hbx upregulate Wnt-5a expression (Liu et al. 2008) and induce nuclear β -catenin. One of the nonstructural proteins of HCV, NS5A, stabilises β -catenin by activating PI3K (Street et al. 2005). Mechanically, NS5A interacts with the SRC homology 3 (SH3) domains of members of the SRC family of tyrosine kinases and modulates kinase activity. Finally CagA of *H. pylori* physically interacts with E-cadherin, and this interaction impairs the complex formation between E-cadherin and β -catenin, causing cytoplasmic and nuclear accumulation of β -catenin (Murata-Kamiya et al. 2007). Additional pathways, including those that are mediated by the transactivation of EGFR (Polk and Peek 2010) or PI3K/AKT (Suzuki et al. 2009; Sokolova et al. 2008; Nakayama et al. 2009), have been demonstrated to regulate β -catenin activation in response to *H. pylori* infection. Activation of PI3K and AKT leads to the phosphorylation and inactivation of glycogen synthase kinase 3 β (GSK3 β), permitting β -catenin to accumulate in the cytosol and nucleus. All these examples demonstrate that by enhancing nuclear β -catenin expression, cancer-causing pathogens are able to induce cell proliferation on one hand and disrupt cell–cell adhesion and induce EMT on the other. Thus, the ability of cancer-causing pathogens to directly target and perturb cell polarity and cell–cell adhesion makes them potent inducers of EMT, which is likely to contribute to their oncogenic properties.

7.3 Cell Polarity: The Last Line of Defence Against Cancer Cell Invasion

Correct establishment and maintenance of cell polarity are required for the development and homeostasis of all metazoans. Reduced expression of components or regulators of cell polarity in human cancers has been reported and reviewed extensively, supporting a tumour-suppressive role of cell polarity (Royer and Lu 2011; Muthuswamy and Xue 2012). Importantly, loss of normal cell polarity and tissue architecture is a defining characteristic of cancer malignancy. Malignant transformation can be induced by the abnormal activation of various oncogenic and growth factor signalling pathways, which not only stimulate cell proliferation but also result in disruption of apical–basal polarity, cell–cell adhesion and EMT. The co-operation between the loss of cell polarity and oncogene activation resembles the actions of cancer-causing pathogens since many oncoproteins of cancer-causing pathogens can disrupt cell polarity on one hand and activate oncogenic signalling on the other. Therefore, we will focus on the evidence supporting a positive role of cell polarity in defending cancer cell invasion.

7.3.1 *Enhanced Growth Factor Signalling Targets Cell Polarity Complex to Induce EMT and Cancer Metastasis*

Deregulation of growth factor signalling such as an elevation of transforming growth factor β (TGF β) signalling promotes EMT during normal development and tumour progression (Thiery 2003). This is partly achieved by the ability of TGF β signalling to target Par6, a component of the apical cell polarity complex (Fig. 7.2). Type II TGF β receptor, TGF β RII, is a receptor tyrosine kinase, which binds and phosphorylates Par6. Phosphorylated Par6 is required for TGF β to induce EMT in mammary gland epithelial cells, and it is also required for Par6–Smurf1 interaction. Smurf1 is an E3 ubiquitin ligase, and it targets the small guanosine triphosphatase (GTPase) RhoA for degradation. RhoA is crucial for the maintenance of the actin cytoskeleton and stabilisation of AJCs. Thus, activation of TGF β signalling ultimately results in the destabilisation and loss of AJCs and the initiation of EMT (Ozdamar et al. 2005). In addition, TGF β signalling activation induces the expression of three families of transcription factors: the Snail, ZEB and bHLH families, either through a Smad-dependent mechanism (in the case of Snail proteins) or indirectly through activation of other transcription factors or relief of repression (Xu et al. 2009). Upon activation, these transcription factors in turn repress epithelial marker gene expression and concomitantly activate mesenchymal gene expression (Peinado et al. 2007).

Abnormal activation of the receptor tyrosine kinase ErbB2 (also known as human epidermal growth factor receptor 2, HER2 or Neu), an oncogene that has

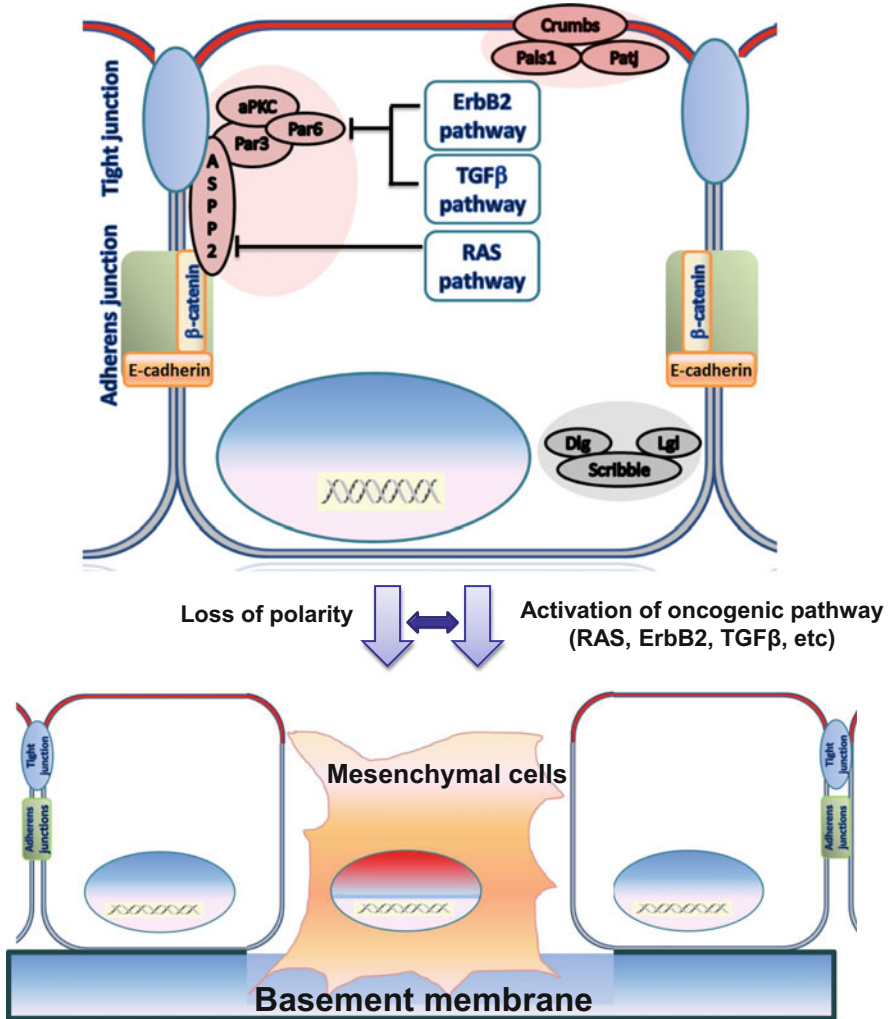


Fig. 7.2 Cell polarity is the last line of defence against cancer cell invasion. Loss of cell polarity co-operates with activation of oncogenes to facilitate epithelial mesenchymal transition (EMT) and to promote cancer cell invasion and metastasis. Activation of TGFβ signalling results in phosphorylation of Par6, and this is required for TGFβ-dependent EMT. Activation of ErbB2 signalling results in disruption of the apical Par6–Par3–aPKC polarity complex by promoting dissociation between Par3 and Par6–aPKC. This function is crucial for ErbB2-mediated disruption of cell polarity. RAS activation induces ASPP2 translocation from cell–cell junctions to the cytosol and nucleus. *T-junctions* (⊥) indicate inhibition

been implicated in human breast, ovarian, gastric, oesophageal and endometrial cancers, can directly disrupt cell polarity and inhibit apoptosis by binding to the Par6–aPKC protein complex (Aranda et al. 2006) (Fig. 7.2). Inhibition of this association restores correct cell polarity and abrogates the anti-apoptotic effects

of ErbB2 but does not affect its role in the stimulation of cell proliferation suggesting that cell polarity may be linked to apoptotic functions. Again, enhanced ErbB2 signalling promotes EMT and cancer invasion. Interestingly, ErbB2 is known to co-operate with mutant p53 to increase tumourigenesis in mice (Li et al. 1997). Furthermore in breast cancers, mutant p53 status in combination with high ErbB2 expression is associated with a very poor prognosis (Rahko et al. 2003). However, reactivation of wild-type p53 by nutlin3 (through inhibition of MDM2) both normalised the sphere-forming activity of the ErbB2-induced mammary tumour cells and concomitantly reduced their tumour-initiating activity (Cicalese et al. 2009).

7.3.2 Loss of Cell Polarity and Activation of RAS Oncogene Induce EMT and Cancer Metastasis

Loss of cell polarity when combined with activation of signals such as RAS oncogene may promote the formation of metastatic tumours (Pagliarini and Xu 2003; Igaki et al. 2006; Brumby and Richardson 2003; Langton et al. 2007, 2009) (Fig. 7.2). For example, Scrib-deficient mutants co-operate with oncogenes to mediate transformation in *Drosophila*. Normally, Scrib-deficient mutant clones, in the eye imaginal discs, are eliminated by c-Jun N-terminal kinase (JNK)-dependent apoptosis. However, in the presence of activated oncogenic pathways such as RAS or Notch, apoptosis is inhibited and neoplastic tumours occur (Brumby and Richardson 2003). Again in *Drosophila*, oncogenic RAS, when expressed within clonal patches of tissue in the eye disc, induces hyper-proliferation, but the transformed cells do not invade into other tissues. Cells lacking components of polarity complex, such as dASPP, Scribble, Dlg, Lgl and Bazooka (equivalent to human Par3), are disorganised in the affected tissue but they also do not invade. However when combined with oncogenic RAS, these defects promote formation of metastatic tumours (Pagliarini and Xu 2003; Igaki et al. 2006; Brumby and Richardson 2003; Langton et al. 2007, 2009). Some of the observations in *Drosophila* were confirmed in mammals recently. Loss of Par3 in primary mammary epithelial cells (MECs) co-operates with oncogenic HRAS to promote tumourigenesis in mouse (McCaffrey et al. 2012). E-cadherin was almost undetectable in Par3-depleted tumours in the presence of activated RAS oncogene (McCaffrey et al. 2012). Additionally, KRAS activation and Scrib loss co-operate to facilitate prostate tumour progression (Pearson et al. 2011). Pearson and colleagues generated male mice in which Scrib loss and hyperactivated KRAS (LSL-KRAS *G12D*) were specifically induced in the prostate (Pearson et al. 2011). It was demonstrated that Scrib loss and oncogenic KRAS co-operate to accelerate disease progression in mice, illustrating the multistep nature of prostate cancer progression and providing evidence to support published studies on *Drosophila* in vivo and mammalian cells in vitro (Dow et al. 2008; Brumby and Richardson 2003).

Consistent with the notion that loss of cell polarity is a hallmark of epithelial cancers, expression of Par3 was significantly reduced in primary oesophageal squamous cell carcinoma (ESCC) compared with their non-tumour counterparts. This reduced expression was associated with positive lymph node metastasis and poor differentiation (Zen et al. 2009). Interestingly, ASPP2, a binding partner of Par3, is often downregulated in metastatic tumours. Reduced ASPP2 expression associates with poor prognosis in diffuse large B-cell lymphomas (Aranda et al. 2006), and ASPP2 expression is reduced in both invasive and metastatic cells compared with normal breast epithelium (Sgroi et al. 1999) and squamous cell carcinomas (SCCs) of the head and neck (Tordella et al. 2013). Importantly, a reduction in ASPP2 expression is sufficient to cause spontaneous development of poorly differentiated SCC in mice in vivo with some of the SCC exhibiting an invasive phenotype (Tordella et al. 2013). This is in agreement with a recent finding that ASPP2 is a molecular switch of EMT and its reverse mesenchymal to epithelial transition (MET), and an inhibitor of metastasis. This newly identified property of ASPP2 requires its ability to bind Par3 and β -catenin (Wang et al. 2014). Additionally activation of the RAS oncogenic signalling pathway, due to a mutation, is a common event in human SCC. It is therefore tempting to speculate that reduced Par3 and ASPP2 expression may co-operate with RAS oncogene activation to promote EMT, cancer invasion and cancer metastasis. Together, the existing evidence suggests that cell polarity may act as the last line of defence against cancer cell invasion.

7.4 Conclusions and Perspectives

The identification of increasing numbers of components or regulators of cell polarity complex as direct pathogenic targets argues strongly that cell polarity is likely to act as a first line of defence to guard us against infection. It is now emerging that components or regulators of cell polarity are binding partners of cellular oncogenes and tumour suppressors. They are also common cellular targets of cancer-causing pathogens. Loss of cell polarity often associates with oncogene activation in highly invasive and metastatic human cancers. This resembles the action of cancer-causing pathogens such as *H. pylori*, HPV and hepatitis viruses that are potent inducers of EMT and cancer invasion. All of these argue for a role of cell polarity as a last line of defence against cancer cell invasion. Future studies are needed to provide experimental evidence to demonstrate precisely how cell polarity acts as a barricade against infection and cancer cell invasion. Finally, cancer-causing pathogens will induce the inflammatory response. Cell remodelling and cell migration are fundamental cellular responses to inflammatory signalling. Therefore, future studies are also needed to examine the interplay between cell polarity and inflammation and cancer metastasis.

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