E-cadherin–integrin crosstalk in cancer invasion and metastasis

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Summary

E-cadherin is a single-pass transmembrane protein that mediates homophilic cell–cell interactions. Tumour progression is often associated with the loss of E-cadherin function and the transition to a more motile and invasive phenotype. This requires the coordinated regulation of both E-cadherin-mediated cell–cell adhesions and integrin-mediated adhesions that contact the surrounding extracellular matrix (ECM). Regulation of both types of adhesion is dynamic as cells respond to external cues from the tumour microenvironment that regulate polarity, directional migration and invasion. Here, we review the mechanisms by which tumour cells control the cross-regulation between dynamic E-cadherin-mediated cell–cell adhesions and integrin-mediated cell–matrix contacts, which govern the invasive and metastatic potential of tumours. In particular, we will discuss the role of the adhesion-linked kinases Src, focal adhesion kinase (FAK) and integrin-linked kinase (ILK), and the Rho family of GTPases.

This article is part of a Minifocus on Adhesion. For further reading, please see related articles: 'Cadherin adhesome at a glance' by Ronen Zaidel-Bar (*J. Cell Sci.* **126**, 373-378). 'Cycling around cell–cell adhesion with Rho GTPase regulators' by Jessica McCormack et al. (*J. Cell Sci.* **126**, 379-391). 'Mechanosensitive systems at the cadherin–F-actin interface' by Stephan Huveneers and Johan de Rooij (*J. Cell Sci.* **126**, 403-413).

Key words: E-cadherin, Integrins, Cancer, Invasion

Introduction

Understanding the processes by which tumour cells invade and metastasise to distant sites (and how to target them), is one of the great challenges in cancer research, as metastatic spread is responsible for ~90% of cancer-related mortality. Tumour cell invasion and metastasis is a complex process that involves multiple steps, including local migration and invasion, dissemination of malignant tumour cells through the lymphatic or haematogenous systems, and the resulting growth or colonization of micro-metastatic lesions and their development into macro-metastases. In common with other 'hallmarks' of cancer (Hanahan and Weinberg, 2011), understanding and inhibiting invasion and metastasis are complicated by the multiplicity of underlying mechanisms, the plasticity of cancer cell behaviour and the evolving nature of the microenvironment. One trait that underpins the ability of cancer cells to metastasise is their ability to change the way in which they interact with the surrounding ECM and with adjacent tumour and stromal cells. E-cadherin is a key mediator of cell–cell adhesions in epithelial tissues, and loss of E-cadherin can promote invasive and metastatic behaviour in many epithelial tumours (Birchmeier and Behrens, 1994). However, it is clear that tumour cells can invade with fully intact and functional cell–cell adhesions as collective groups of cells, and that a loosening of cell–cell contacts is sufficient to permit this collective migration and invasion. This requires coordination of cues from the surrounding tumour environment, to regulate both cell–cell and cell–ECM interactions. Here, we review the role of E-cadherin in tumour cell invasion and metastasis, with particular emphasis on the interplay between E-cadherin and cell–ECM interactions that are mediated by integrin matrix receptors. We discuss the key signalling intermediates that regulate this crosstalk, as well as recent work that supports a physical interaction between integrin- and E-cadherin-mediated adhesions, which governs E-cadherin adhesive strength and cell migration.

E-cadherin and adherens junctions

E-cadherin is the prototypical member of the type-1 classical cadherins and is found at adherens junctions (AJs), structures that mediate cell–cell interactions. It was first discovered as a Ca^{2+}-dependent cell surface protein that mediated cell–cell adhesion in early mouse embryo blastomeres (Hyafil et al., 1981; Hyafil et al., 1980). Targeted knockout of the gene encoding E-cadherin in the mouse is embryonic lethal, as the embryos cannot generate an epithelium, which is required for the development of multicellular organisms (Larue et al., 1994; Riethmacher et al., 1995).

E-cadherin is a single-pass transmembrane glycoprotein containing five extracellular repeats that mediate its Ca^{2+}-dependent homophilic interaction with opposing molecules on neighbouring cells (reviewed by van Roy and Berx, 2008). The cytoplasmic domain of E-cadherin binds to members of the catenin protein family, namely β-catenin and p120-catenin, which act to link the multi-protein complex to the actin cytoskeleton through α-catenin; the clustering of cadherin–catenin complexes on adjacent cells then leads to localised actin remodelling that is required for AJs assembly (Fig. 1). Maintenance of AJs is also dependent on the dynamic actin cytoskeleton. Many actin regulators, such as the Arp2/3 complex, Ena/VASP and Wiskott-Aldrich syndrome protein (WASP) family members are localised to AJs, and disruption of their function leads to a loss of junctional integrity, indicating a close interplay between junction dynamics and the actin cytoskeleton (Ratheesh and Yap, 2012). The binding of β-catenin occurs shortly after the synthesis of E-cadherin and acts to chauffeur

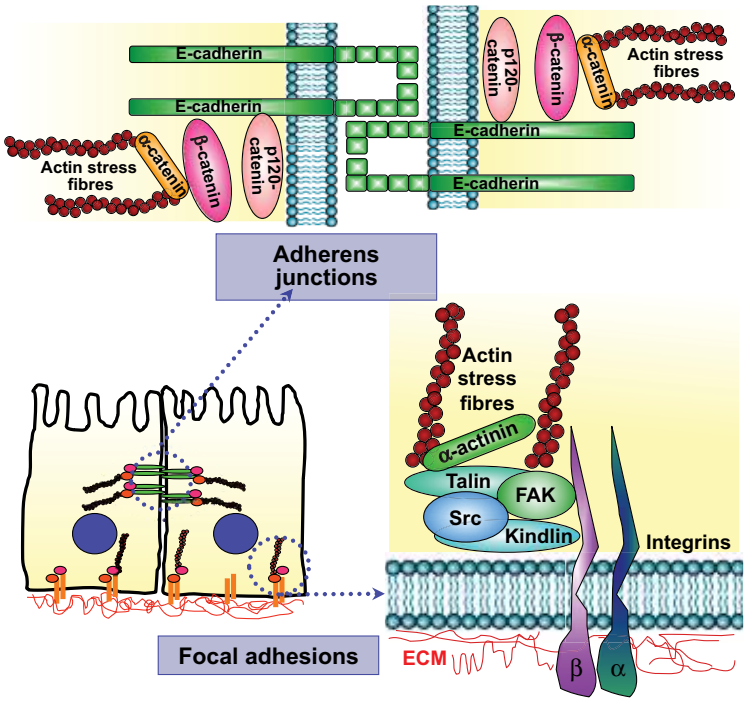

Fig. 1. Schematic representation of E-cadherin-mediated cell–cell adherens junctions and cell–ECM integrin-mediated adhesions. E-cadherin is a single-pass transmembrane protein, whose extracellular domain, which is composed of five Ca^{2+}-binding repeats (green squares), mediates specific homophilic interactions with neighbouring cells. The intracellular domain of E-cadherin associates with catenins, which tether these complexes to the actin cytoskeleton forming stable AJs. Focal adhesions are multi-protein complexes that mediate the contact of cells to the ECM (red lines); the membrane receptors for this type of adhesion are heterodimers of α- and β-integrins. They form multi-protein complexes that are linked to the actin cytoskeleton. Key players that link integrins to the actin cytoskeleton include talin, kindlins and α-actinin. The tyrosine kinases focal adhesion kinase (FAK) and Src are also part of the integrin complexes and are key mediators of signalling downstream of integrins.

E-cadherin to the plasma membrane where they remain in a complex (Chen et al., 1999). By contrast, p120-catenin is reported to stabilise E-cadherin at the plasma membrane (Ireton et al., 2002) by controlling its cell surface levels through the regulation of cadherin trafficking (D'Souza-Schorey, 2005; Davis et al., 2003). For many years, α-catenin was regarded as the direct link between the cadherin–catenin complex and the actin cytoskeleton. However, recently evidence has emerged to suggest that the role of α-catenin is more complex, and involves the regulation of actin-filament dynamics and recruitment of other actin modulators to the cadherin–catenin complex (Scott and Yap, 2006).

E-cadherin membrane dynamics and trafficking

AJs are not static, but rather are highly dynamic structures that undergo rapid and constant remodelling. The bidirectional transport of E-cadherin molecules to and from the membrane supports a local remodelling of cell–cell contacts, and controls both E-cadherin stability at the plasma membrane and the steady-state cellular levels of E-cadherin. E-cadherin undergoes endocytosis, which is controlled by multiple mechanisms, including clathrin- and caveolin-mediated endocytosis (reviewed by Yap et al., 2007). Given the dynamic nature of AJs, several groups have recently utilised new fluorescence imaging techniques that allow tracking of single E-cadherin molecules in real time. Fluorescence recovery after photobleaching (FRAP) and photoactivation have been used to analyse the proportion of mobile molecules within a pool, and the rate at which these molecules move. For instance, a FRAP study in confluent monolayers of cells has highlighted the requirement for endocytosis in the dynamic turnover of E-cadherin at AJs, and in fact, suggested that endocytosis is a major mechanism for E-cadherin exchange at the membrane in some circumstances (de Beco et al., 2009). However, endocytosis is not the only mechanism responsible for controlling the presence of E-cadherin at sites of cell–cell adhesion. Indeed, experiments utilising laser trapping of E-cadherin-coated beads have shown that the initial accumulation of E-cadherin at sites of cell–bead interaction is dependent on active membrane dynamics, and relies on diffusion-mediated trapping of E-cadherin molecules at these sites (Perez et al., 2008). However, E-cadherin does not solely exist as freely diffusible monomers within the membrane. It has been reported that E-cadherin molecules can homodimerise, or oligomerise, into higher-order complexes known as cadherin 'clusters' (Nagar et al., 1996); it is proposed that these clusters can contribute to the strengthening of cell–cell adhesions when they are aligned in a parallel orientation between cells (Yap et al., 1997). Recently, analysis of the molecular dynamics of E-cadherin within such clusters by using FRAP revealed that their contents are highly immobile and that there is relatively little exchange with E-cadherin in the surrounding membrane (Cavey et al., 2008). Furthermore, these clusters do not undergo extensive re-distribution within the membrane owing to constraints imposed by the actin cytoskeleton, suggesting that they represent genuine adhesive structures (Cavey et al., 2008). The use of photobleaching and photoactivation, which allows the tracking of green fluorescent protein (GFP)–E-cadherin within cells, has shown that a fine balance exists between the movement of E-cadherin molecules within, and away from, the cell surface, and that this is important for the modulation of AJs and their adhesive function (Canel et al., 2010a). The dynamics of E-cadherin at AJs is, therefore, of undoubted importance to maintain junction integrity. Despite this, the mechanisms underlying how E-cadherin dynamics contribute to the strength of cell–cell junctions are still largely unknown.

E-cadherin as an invasion and tumour suppressor

E-cadherin expression or its cell surface localisation is often lost in advanced tumours and has been linked, at least in some cases, to a higher incidence of metastasis and tumour recurrence (Berx and van Roy, 2009; Birchmeier and Behrens, 1994). Loss of E-cadherin expression in human tumours is most commonly caused by methylation of its promoter, or upregulation of the

transcriptional repressors SNAIL (also known as SNAI1), SLUG (also known as SNAI2), SIP1 (also known as GEMIN2) and Zeb1, which target the E-cadherin promoter (reviewed by Berx and van Roy, 2009). However, germline mutations in E-cadherin have also been identified and are reported to result in the predisposition of several family generations to familial gastric cancer (Guilford et al., 1998). In general, these mutations are associated with the synthesis of a truncated form of E-cadherin, although an additional mutation in the calcium-binding domain has also been identified. In addition, somatic mutations are found in lobular breast cancer combined with the heterozygous loss of the wild-type human E-cadherin gene (CDH1) (Berx and Van Roy, 2001). Studies using animal models of pancreatic and breast cancer have demonstrated that loss of E-cadherin function is a causal factor in the promotion of invasion and metastasis, largely through the conversion of epithelial tumour cells into highly migratory and invasive cells (Derksen et al., 2006; Perl et al., 1998).

The loss of E-cadherin and the resulting suppression, or weakening, of cell–cell adhesion has been regarded as a crucial step in the epithelial–mesenchymal transition (EMT) process (Kalluri and Weinberg, 2009). EMT is the coordinated de-stabilisation of cell–cell contacts and acquisition of a more migratory and invasive mesenchymal phenotype, together with respective changes in gene expression patterns, and is regarded as a potentially important event in the metastatic spread of tumour cells (reviewed by Christiansen and Rajasekaran, 2006; Guarino et al., 2007). EMT is not a process that is unique to cancer cells, but is a manifestation of normal cell behaviour. Indeed, EMT is required for processes including embryogenesis, organ development and wound repair, and appears to be regulated by similar signalling networks to those that control cancer EMT (reviewed by Thiery, 2002). Although it is widely accepted that cancer cells can undergo EMT to facilitate dissemination from the primary tumour, it is also clear that tumour cell invasion can occur with fully intact and functional cell–cell adhesions, and that a partial transition or loosening of cell–cell contacts might be sufficient to facilitate invasion (Christiansen and Rajasekaran, 2006). This collective movement of cells requires the maintenance of cell–cell adhesions and is utilised during normal morphogenesis, for example during embryonic development (Christiansen and Rajasekaran, 2006). This typically involves the movement of sheets or strands of epithelial cells in a polarised and directional manner (Friedl and Gilmour, 2009).

The mode of invasion, whether as single mesenchymal cells or as collective groups of cells, that is employed by particular tumour types is rarely definite. Instead, advanced cancers can display plasticity with respect to morphological characteristics and the modes of migration and invasion they use; these can be altered and adapted according to interactions with and signals they receive from, the local tumour environment (Gaggioli et al., 2007; Giampieri et al., 2009) (Fig. 2). The key factor that determines the net outcome in terms of invasive migration and its underlying mechanism is the status of and the balance between E-cadherin-mediated AJs and integrin-mediated cell–matrix contacts.

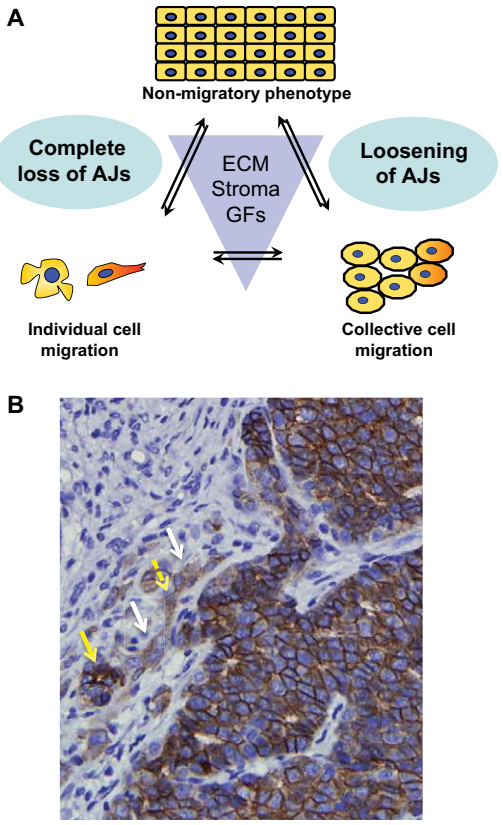

Fig. 2. Plasticity of cancer cell invasion. (**A**) Tumour cell invasion can occur as single cells or as collective groups of cells moving together. Cancer cells display plasticity in the chosen mode of migration, and this is dependent on diverse signalling inputs from the surrounding tumour microenvironment that control the interplay between adhesive structures (Friedl and Alexander, 2011). Both types of invasion are dependent on integrin-mediated adhesion to the ECM, whereas collective invasion also requires the generation and maintenance of dynamic cell–cell adhesions, with loosening of cell junctions being sufficient to permit movement. This can be achieved through downregulation of E-cadherin expression at the cell periphery (dashed yellow arrow in B), or through more subtle changes in E-cadherin dynamics in cells that have strong membranous E-cadherin (yellow arrow in B) (Canel et al., 2010b). (**B**) Image showing the invading edge of a polyomavirus (PyV) middle T (MT) oncogene-induced mouse mammary tumour stained for E-cadherin. Yellow arrows show the collective invasion of cells that have retained E-cadherin staining at the periphery, whereas the white arrows indicate individual cells that have downregulated or internalised E-cadherin, demonstrating that loss of E-cadherin from the cell periphery is not a prerequisite for cell invasion. Localised environmental cues at the invasive front control the changes in E-cadherin that govern the different modes of invasion that cells from a single tumour can adopt. GFs, growth factors.

Integrin-mediated adhesions

Integrins are heterodimeric cell-surface glycoproteins that serve to mediate cell–ECM interactions, thereby linking cues from the extracellular environment to the actin cytoskeleton (Hynes, 2002). These membrane-spanning proteins consist of two subunits, termed α and β, of which there are at least eighteen α-subunits and eight β-subunits. The resulting multitude of possible combinations gives rise to more than 20 different integrins, which act to differentially control a range of biological processes through selective binding to extracellular substrates (Humphries et al., 2006). Integrins transmit signals from the outside to the inside of the cell through the assembly of multi-protein complexes that link integrins to the actin cytoskeleton. These are comprised of structural, adaptor and signalling

proteins, and this complex network of protein interactions has been termed the adhesome, in which 180 protein–protein interactions have been identified to date (Zaidel-Bar and Geiger, 2010). Key players that link integrins to the actin cytoskeleton include talin and kindlins, which are involved in the conformational activation of integrins and vinculin, which acts to strengthen the link with the cytoskeleton and the actin cross-linker α-actinin (Fig. 1). Integrin engagement leads to rapid changes in lipid kinase activity and the activation of the focal adhesion-linked protein tyrosine kinases Src and FAK. This promotes dynamic actin and adhesion changes at the cell membrane, and activation of numerous downstream signalling pathways that have a pivotal function in cellular processes, such as adhesion, migration, proliferation and survival (Cabodi et al., 2010; Legate et al., 2006). Key downstream effectors include the small Rho GTPases, Rho, Rac and CDC42, which coordinate the changes in the actin cytoskeleton that drive cell polarity and migration (Jaffe and Hall, 2005). Initial integrin engagement leads to the formation of short-lived nascent adhesions, which mature into larger focal complexes and then focal adhesions that tether to actin stress fibres. The coordinated assembly and disassembly of these adhesive structures coupled with actomyosin-driven contractility provide the basic mechanics that are required for cell migration (Parsons et al., 2010).

Crosstalk between E-cadherin- and integrin-mediated adhesions

The crosstalk between epithelial cell–cell adhesion and cell–matrix adhesion signalling, and the dynamic interplay between the two, contribute to the plasticity within tumour cells that allows them to respond to external cues, which in turn drives effective migration and invasion. Below, we will review data on the key signalling intermediates that regulate this crosstalk, as well as discuss recent work that is in support of a physical interaction between integrin- and E-cadherin-mediated adhesions that governs the adhesive strength of E-cadherin (Fig. 3).

Physical interactions between integrin- and E-cadherin-mediated adhesions

The crosstalk between integrins and E-cadherin might be mediated by the physical disruption of cell–cell adhesions, which is driven by integrin-induced changes in actomyosin contractility (de Rooij et al., 2005; Martinez-Rico et al., 2010). Actomyosin contractility is regulated by the phosphorylation of myosin II light chain (MLC) by two key kinases, MLC kinase (MLCK, also known as MYLK) and Rho-associated protein kinase (ROCK), a downstream effector of RhoGTPase. These two kinases are pivotal in regulating crosstalk between the two adhesion types by controlling actomyosin contractility and thus adhesive strength, which impacts on the collective migration of cells (Friedl and Gilmour, 2009; Parsons et al., 2010). Interestingly, other signalling proteins, including the Rho GTPases and Src (discussed in detail below), are also important intermediaries in regulating actomyosin contractility. Recently, several biophysical approaches have helped to establish the importance of such physical interactions in the control of adhesion strength and cell migration. For instance, when the cell matrix protein fibronectin is spotted onto substrates surrounded by E-cadherin molecules, cells are unable to simultaneously form both integrin–ECM (fibronectin)- and E-cadherin-mediated adhesions (Tsai and Kam, 2009). However,

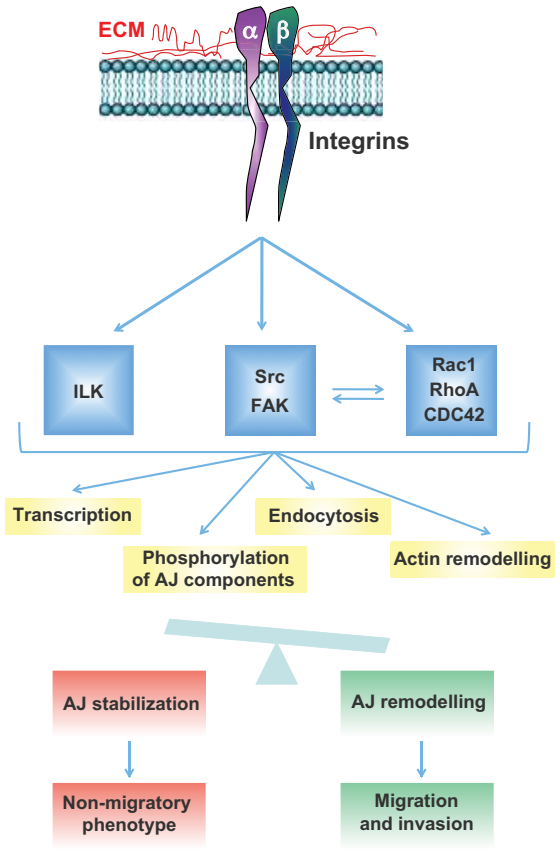

Fig. 3. Examples of key mediators of integrin signalling that regulate E-cadherin-mediated adhesions. Integrin engagement triggers several signalling cascades including those that are mediated by ILK, FAK and Src and Rho GTPases, such as Rac1, RhoA and CDC42. These signaling pathways are not independent but linked; for instance, Src is required for inactivation of RhoA downstream of integrin activation (Arthur et al., 2000), whereas RhoGTPases direct the specific intracellular targeting of Src (Timpson et al., 2001). As discussed in the main text, this signalling network downstream of integrins leads to diverse cellular responses, including changes in actin dynamics, which regulate AJs. In addition, activation of these pathways leads to changes in the transcriptional and post-transcriptional control of AJ components and the control of E-cadherin endocytosis. The net input from these signalling pathways can, therefore, shift the balance between stabilization or remodelling of AJs, which ultimately governs the migratory capacity of epithelial cells.

this suppression of AJ formation by integrin engagement appears to be rigidity dependent, with 'softer' substrates being permissive for the co-assembly of AJs and integrin adhesions (Tsai and Kam, 2009). In a similar manner, the use of microprinted fibronectin patterns and microbeads that are coated with the E-cadherin extracellular domain showed a strong negative feedback between the ability of cells to form integrin dependent cell–ECM and E-cadherin mediated cell–cell interactions (Al-Kilani et al., 2011). In addition, Borghi and colleagues utilised micropatterned surfaces that comprise alternating stripes of ECM components (collagen IV) and adjustable amounts of E-cadherin molecules, and analysed the motility of epithelial cells in response to combinations of cell–ECM and E-cadherin-mediated cell–cell adhesion cues by traction force microscopy (Borghi et al., 2010). They show that E-cadherin can regulate both lamellipodia

activity and the distribution of focal adhesions, and can control the directionality of cell migration, but not the rate of cell movement. They also identified two pools of α-catenin; a membrane-associated pool that is required for E-cadherin-mediated adhesion and downregulation of lamellipodia activity, and a cytosolic pool that downregulates the migration rate in an E-cadherin adhesion-independent manner. Although the molecular mechanisms are yet to be defined, this suggests that α-catenin is an important regulator of both E-cadherin adhesion and cell migration, and has a pivotal role in the crosstalk between E-cadherin adhesions and integrin-mediated cell–ECM interactions (Borghi et al., 2010).

Signalling intermediates regulating crosstalk

There is a considerable body of evidence that links signals downstream of integrin–ECM adhesions to the regulation of E-cadherin-mediated adhesions. Here, we discuss recent data on the role of the non-receptor tyrosine kinases Src and FAK, ILK and small GTPases, and highlight how this crosstalk influences the collective migration, invasion and metastatic potential of tumour cells.

Src and FAK

Src and FAK have key functions in the transmission of signals downstream of integrin activation (Cabodi et al., 2010; Mitra and Schlaepfer, 2006). Although the precise mechanisms through which signals that originate from integrin activation at the cell surface lead to activation of Src and FAK remain unclear, the activation of both kinases requires the release of auto-inhibitory protein conformations that result in increased kinase activity. For FAK, this permits the binding of Src to the autophosphorylation site of FAK, leading to Src-mediated phosphorylation of tyrosine residues in the kinase loop of FAK and its full catalytic activation (for reviews on regulation of Src and FAK activity, see Frame et al., 2010; Superti-Furga and Gonfloni, 1997). Interestingly, Src and FAK localise to both integrin-mediated focal adhesions and to cell–cell contacts, which raises important unanswered questions of whether these pools are interchangeable, and what controls the spatial regulation and activation of Src and FAK in response to cues that regulate cell–cell adhesion dynamics.

The role of Src and FAK in controlling AJs is complex, because they can regulate both AJ assembly and disassembly. It has been known for some time that Src can phosphorylate components of the AJ, and that this has a role in the dynamic behaviour and normal turnover and assembly of AJs (Calautti et al., 1998; McLachlan et al., 2007; Owens et al., 2000); more recently, a role for FAK in AJ formation has also been demonstrated (Playford et al., 2008). However, in contrast to the tightly regulated activity of the proto-oncogene Src found in normal cells, expression of oncogenic Src, which contains mutations in the regulatory carboxy terminal tail, leads to constitutive activation of the kinase and the phosphorylation of AJ components, which is generally associated with disruption of cell–cell adhesions and increased invasiveness (Behrens et al., 1993; Matsuyoshi et al., 1992). A similar role for FAK in the disruption of AJs has also been established; here, phosphorylation of β-catenin by FAK results in a loss of AJ integrity following integrin engagement (Koenig et al., 2006), and phosphorylation of vascular endothelial (VE)-cadherin by FAK in endothelial cells upon stimulation with vascular endothelial growth factor (VEGF) also results in the disruption of cell–cell adhesions (Chen et al., 2012). Tyrosine phosphorylation alters the affinity of components of the AJs for their binding partners, resulting in a weakening or severing of the cadherin–cytoskeletal link, and might result in the disruption of AJs in tumours, in which Src and/or FAK activity is constitutively high. However, a transient activation of Src and/or FAK by either integrin activation or growth factor stimulation might also lead to phosphorylation of AJ components and the disruption of junctions, and could have a pivotal function in coordinating signals that originate not only from the ECM through integrins but also from growth factors to regulate AJ integrity. Further support for an integral role of FAK and Src in assimilating multiple signal inputs that control AJs is the observation that the FAK–Src pathway regulates transforming growth factor β (TGFβ)-induced EMT (Cicchini et al., 2008) and is also involved in coupling integrin activation with TGFβ-induced EMT, which has been linked to a more invasive and metastatic phenotype (Bhowmick et al., 2001; Galliher and Schiemann, 2006; Wendt and Schiemann, 2009).

As well as direct phosphorylation of AJ components, Src and FAK can also regulate AJs by controlling the stability of E-cadherin protein levels (Cicchini et al., 2008; Coluccia et al., 2006). Although the underlying mechanisms are not well understood, there are reports that Src can control expression of E-cadherin through transcriptional control of the E-cadherin promoter (Menke et al., 2001). In addition, recently, an increased expression of E-cadherin has been observed in FAK-null mouse embryonic cells, which was attributed to the regulation of the E-cadherin transcriptional repressor SNAIL1 (Li et al., 2011). This increase in E-cadherin expression is associated with the assembly of AJs and the conversion to an epithelial phenotype.

In addition to regulating the absolute levels of E-cadherin within the cell, Src and FAK can also regulate the endocytosis of E-cadherin and thereby its membrane localisation. By fine-tuning the dynamic turnover of E-cadherin at the cell membrane, Src and FAK can control the strength of AJs, which in turn impacts on the migratory and invasive capacity of tumour cells. Early studies showed that oncogenic Src could control the endocytosis of E-cadherin (Fujita et al., 2002; Palacios et al., 2005). However, a more subtle regulation of the control of E-cadherin internalisation by Src was shown to depend on integrin signalling (Avizienyte et al., 2002). Furthermore, the resulting disruption of AJs was shown to require Src-dependent phosphorylation of FAK and the formation of a FAK–Src complex, which is required for full activation of FAK (Avizienyte et al., 2004). More recently, we found that small interference RNA (siRNA)-mediated depletion of FAK, or β1-integrin, inhibits E-cadherin endocytosis and is associated with a strengthening of cell–cell adhesions and reduced collective invasion (Canel et al., 2010b), whereas pharmacological inhibition of FAK or Src decreases the collective movement of tumour cells *in vivo*, which correlates with changes in the turnover of E-cadherin at the cell membrane (Canel et al., 2010b). Importantly, these studies show that the fine control of E-cadherin dynamics by the integrin–Src–FAK axis can have a major impact on the behaviour of epithelial tumour cells, both *in vitro* and *in vivo*, and that the complete loss of cell–cell junctions is not required for the acquisition of a motile and invasive phenotype.

So what are the signals downstream of Src and FAK that regulate AJs? Src can cooperate with other kinase signalling pathways, such as mitogen activated protein kinase (MAPK),

MLCK and ROCK, to promote the peripheral accumulation of phospho-myosin, which is required to maintain a mesenchymal phenotype and inhibit the formation of E-cadherin mediated cell–cell adhesions (Avizienyte et al., 2004). Therefore, the activity of peripheral phospho-myosin and the regulation of contractile force can act as a point of convergence for upstream signals that regulate integrin and E-cadherin-mediated adhesions (Avizienyte et al., 2004). Another pathway that has been implicated in acting downstream of the integrin–Src–FAK axis is the Wnt–β-catenin pathway (Coluccia et al., 2006; Koenig et al., 2006). Here, activation of the integrin–Src–FAK axis can lead to the nuclear translocation of β-catenin following disruption of AJs, and the subsequent activation of its transcriptional activity (Coluccia et al., 2006; Koenig et al., 2006) that is commonly associated with more invasive and aggressive tumours (Schmalhofer et al., 2009).

Although, the mechanisms and signalling events involving the Src–FAK axis are not yet fully understood, and are undoubtedly dependent on the context because cells receive varied signals from the surrounding microenvironment, the control of both E-cadherin-mediated and integrin-mediated adhesions by Src and FAK places them at the heart of a crosstalk between these two adhesion types and can provide cells with a pro-invasive advantage. Targeting the Src and FAK kinase activities therefore provides a promising approach to inhibit not only the dissemination of tumour cells that have undergone EMT, but also of those cells that have retained cell–cell junctions, and that migrate and invade in a collective manner.

ILK

ILK is a serine/threonine protein kinase that interacts with the cytoplasmic domains of β-integrin subunits. It has been linked to signalling downstream of integrins, and connects integrin-mediated responses to actin changes (reviewed by Legate et al., 2006). However, there is also a considerable body of evidence to suggest that overexpression of ILK is involved in the downregulation of E-cadherin expression, and an induction of EMT and a more invasive phenotype (Oloumi et al., 2004). The inhibition of E-cadherin transcription is thought to be the key mechanism by which ILK regulates the expression of E-cadherin (reviewed by Oloumi et al., 2004); this can be mediated by the SNAIL and ZEB families of E-cadherin transcriptional repressors (Matsui et al., 2012; McPhee et al., 2008; Tan et al., 2001). The localisation of ILK at integrin adhesions places it at a central position for coordinating the signals from growth factors and integrins that control both matrix assembly and E-cadherin expression. In keratinocytes, ILK has also been found at cell–cell adhesions, where it has a function in the early stages of AJ formation (Vespa et al., 2005; Vespa et al., 2003). ILK might therefore act as a rheostat to control normal epithelial behaviour, but this fine control could be lost when it is overexpressed in tumours, resulting in the induction of EMT, and a more invasive and aggressive tumour phenotype.

Small GTPases

Although the small Rho GTPases, CDC42, Rac1 and RhoA are best known for their role in regulating the actin cytoskeleton and directed cell migration (Ridley, 2000), there is also a substantial body of evidence implicating these small GTPases in the regulation of cell–cell adhesions (reviewed by Lozano et al., 2003; Menke and Giehl, 2012). Here, we provide an overview of some of the studies that have contributed to the compelling evidence that links Rho GTPases to AJ regulation. It has been suggested that the homophilic interaction of E-cadherins is the first step in AJ assembly, and that the lamellipodium formation, which is triggered by Rac1, can facilitate this initial cell–cell contact by placing E-cadherin molecules in the proximity of adjacent cells (Ehrlich et al., 2002). Once contacts are established, the concerted action of Rho and Rac regulates the stabilization of cadherin-mediated adhesions (Braga et al., 1999; Braga et al., 1997). However, it is clear that multiple signalling pathways downstream of Rac and Rho can regulate AJs, leading to either stabilisation or disruption of junction integrity. The predominant pathway might depend on activity levels, expression of particular family members, cell type or even cell substrate. For example, Sahai and Marshall have identified two downstream effectors of RhoA with opposing effects on AJs: mDia-dependent actin polymerisation stabilises AJs, whereas ROCK-mediated actomyosin contractility disrupts AJs (Sahai and Marshall, 2002). Interestingly, small Rho GTPases have also been shown to regulate AJs by mechanisms that do not involve a direct effect on the actin cytoskeleton. For instance, CDC42 has been reported to be crucial for the disassembly of AJs, and the acquisition of a mesenchymal and more migratory phenotype (Shen et al., 2008). In this study, Ca^{2+} depletion not only weakens AJs but also leads to CDC42 activation, which in turn triggers the epidermal growth factor receptor (EGFR)-dependent stimulation of Src, resulting in E-cadherin phosphorylation. Moreover, the disruption of cell–cell contacts by endocytosis of E-cadherin has also been reported to occur upon activation of Rac1 (Akhtar and Hotchin, 2001), and it is very likely that Rho GTPases cooperate with other small GTPases of the ADP ribosylation factor (Arf) and Rab families, which regulate membrane trafficking to control cell migration and invasion (Ramsay et al., 2007).

So far, most studies exploring the interplay between integrins and cadherins have focused on the regulation of cell–cell contacts by integrin adhesions. However, recent evidence has highlighted the potential bi-directionality of this crosstalk, whereby AJs can also regulate integrin-mediated adhesions (Balzac et al., 2005). Rap1, a member of the Ras subfamily of small GTPases is involved in the control of integrin activation and is also crucial for both *de novo* formation and remodelling of AJs (for reviews, see Bos, 2005; Retta et al., 2006). Balzac and co-workers observed a strong activation of Rap1 upon AJ disassembly, which was associated with the formation of integrin-mediated focal adhesions placing Rap1 at a key interface between these two adhesion types (Balzac et al., 2005).

Finally, it is worth mentioning that cadherin-mediated adhesion also modulates the activity of other small GTPases (Arthur et al., 2002). Thus, small GTPases act to coordinate signals that are triggered by extracellular cues, either through cell–cell or cell–ECM interactions, and orchestrate the subsequent changes in the actin cytoskeleton, which are essential to control directional cell migration and invasion.

Conclusions and perspectives

The function of E-cadherin in tumour progression is undoubtedly complex; however it is such a crucial player in cancer phenotypes that continuing efforts to understand its mode of regulation and function are vital. Although most of the evidence implicates the loss of E-cadherin with EMT and the acquisition of a more invasive and metastatic phenotype, it is clear that loss of

E-cadherin is not always a prerequisite for tumour progression. This highlights the multiplicity of tumour cell mechanisms and their ability to adapt to local evolving environmental cues to allow local invasion and often the ultimate outgrowth at metastatic sites.

Much of the research carried out to date provides only a snapshot of a highly dynamic process, in which the interplay between integrin- and E-cadherin-mediated adhesions is in constant flux. Endocytosis has a key role in controlling the dynamics of E-cadherin-mediated cell–cell adhesions, but the same pathways are involved in the trafficking of integrins, which also acts to control their availability at the plasma membrane and thus their adhesive capacity (Bridgewater et al., 2012). Many of the Rab and Arf GTPases that control this trafficking have been shown to be deregulated in tumours, and their activity can influence the ability of cells to migrate and invade (Bridgewater et al., 2012; Ramsay et al., 2007). The deregulation of endocytic and recycling pathways is considered an up-and-coming hallmark in cancer, because the functionality not only of integrin and cadherin adhesions but also growth factors is compromised by aberrant vesicular transport (Mosesson et al., 2008). How such trafficking impacts on and/or controls the crosstalk between integrin and E-cadherin-mediated adhesions remains to be established, but it might provide a new pathway to target the spread of cancer.

The development of new intravital imaging technologies and the use of complex genetic mouse models for cancer will enable the tracking and monitoring of E-cadherin and integrin pools. Moreover, this will allow the monitoring of cancer cell behaviour deep inside tumour tissue, including the interaction and relationship with the host stroma (Gaggioli et al., 2007; Wyckoff et al., 2011; Wyckoff et al., 2007), as well as in the future, also with components of the innate immune system. Such technologies are crucial as we move forward to identify potential new anti-metastatic therapeutics, and test their effectiveness (Canel et al., 2010b; Serrels et al., 2009). The most advanced of these are small-molecule inhibitors of Src, which are currently being tested extensively in the clinic in a number of solid tumour types. However, the results have been disappointing, and further work is required to determine whether the ability of these inhibitors to block the migratory and invasive capacity of cells can be harnessed to provide clinical benefit (Creedon and Brunton, 2012).

The crosstalk between cell–cell and cell–matrix adhesions can be governed by a number of signalling pathways that impact on the migratory and invasive capacity of cells through the disruption of AJs when integrins are activated. But what are the molecular pathways that are activated downstream of E-cadherin loss? Disruption of AJs can lead to the nuclear translocation and increased transcriptional activity of β-catenin (Coluccia et al., 2006; Koenig et al., 2006). However, loss of E-cadherin in mouse models is not sufficient to activate β-catenin signalling (Herzig et al., 2007; Schackmann et al., 2011), and it appears likely that the ability of E-cadherin to bind β-catenin at the AJ leads to fine-tuning of signalling through the canonical Wnt–β-catenin pathway (Jeanes et al., 2008). In addition, loss of E-cadherin also induces a number of transcription factors that act independently of β-catenin to influence tumour progression (Onder et al., 2008). Loss of E-cadherin can also affect growth factor signalling (Jeanes et al., 2008), and a more complete understanding of the consequences of E-cadherin loss on downstream signalling is required before we can begin to develop strategies that might reverse or prevent the acquisition of a more invasive and metastatic phenotype that is associated with E-cadherin loss. Global proteomic analysis of signalling pathways and networks upon interventions that perturb or promote E-cadherin-mediated adhesion, will allow a greater understanding of biochemical events that are triggered by E-cadherin function and that might be important in epithelial cell plasticity and crosstalk between key types of adhesions.

Although we have focussed here on the role E-cadherin has in suppressing a more motile and invasive phenotype, the downstream consequences of reduced E-cadherin functionality can impact on a number of other tumour-associated phenotypes, such as proliferation, survival, angiogenesis and colonization at metastatic sites (Derksen et al., 2006; Ma et al., 2010; Onder et al., 2008; Wendt et al., 2011). Of interest is the emerging role of p120-catenin, which controls both E-cadherin stability and also the activity of RhoGTPases (Menke and Giehl, 2012). Cytosolic p120-catenin resulting from loss of E-cadherin controls tumour growth and survival through the regulation of Rho GTPase signals and the interplay with integrin survival signals, and highlights the complexity of indirect crosstalk between adhesion types that can influence tumour cell behaviour (Schackmann et al., 2011). Moreover, E-cadherin expression has been linked to drug resistance, and clinical trials of the EGFR inhibitor erlotinib in non-small-cell lung cancer have revealed better responses in patients with high E-cadherin expression (Voulgari and Pintzas, 2009), although it remains to be established whether E-cadherin is merely a biomarker of response or whether it participates in the mechanism of drug resistance.

In this Commentary we have highlighted crosstalk mediated by Src, FAK, ILK and RhoGTPases (Fig. 3). However, there is also evidence to suggest that a number of other signalling intermediates might also have a function in this crosstalk. For example, a number of proteases, such as calpains and metalloproteinases, which are regulated by integrins, can cleave E-cadherin and thus impact on AJ integrity (Noë et al., 2001; Rios-Doria et al., 2003). The interplay between AJs and the actin cytoskeleton is also a key factor in the regulation of junctional homeostasis. Actin regulators, such as the Ena/VASP and WASP family of proteins and cortactin can modulate AJs (Ratheesh and Yap, 2012), and how they link to changes in integrin ligation that can impact on E-cadherin function remains to be established. Interestingly, both cortactin and WASP family proteins can be phosphorylated by Src, indicating a further level of complexity in the network of signalling pathways that control AJs. Furthermore, the crosstalk between E-cadherin-mediated cell–cell adhesions and integrin-mediated cell–matrix contacts is regulated by signalling mechanisms that are still being uncovered. For example, recent findings point towards reactive oxygen species as emerging candidates for modulating the crosstalk between integrin adhesions and AJs (reviewed by Goitre et al., 2012), as well as the Hippo signalling pathway (Kim et al., 2011). It is important to more fully understand how the molecular events downstream of adhesion dynamics and crosstalk orchestrate tumour progression and survival, as well as the response to therapy, so that new therapeutic strategies can be devised to target metastatic disease, thus meeting unmet clinical needs of refractory advanced cancers (Valastyan and Weinberg, 2011).

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