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Actin cytoskeletal control during epithelial to mesenchymal transition: focus on the pancreas and intestinal tract

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The formation of epithelial tissues allows organisms to specialise and form tissues with diverse functions and compartmentalised environments. The tight controls on cell growth and migration required to maintain epithelia can present problems such as the development and spread of cancer when normal pathways are disrupted. By attaining a deeper understanding of how cell migration is suppressed to maintain the epithelial organisation and how it is reactivated when epithelial tissues become mesenchymal, new insights into both cancer and development can be gained. Here we discuss recent developments in our understanding of epithelial and mesenchymal regulation of the actin cytoskeleton in normal and cancerous tissue, with a focus on the pancreas and intestinal tract.

Epithelia are highly organised sheets of cells that serve to form a barrier between external and internal spaces in tissues. They are important for the formation of tubes and the creation of a luminal space where the internal environment can be rendered distinct from the outside world. Epithelial specialisation arose when eukaryotic organisms committed to being multicellular and having functionally specialised tissues, rather than just growing as colonies of more or less identical clonal cells. Epithelial cells are polarised with respect to top and bottom, as well as within the plane of the tissue. Epithelial cells form junctions with their neighbours, involving specialised cytoskeletal protein assemblies. While metazoans have the clearest commitment to epithelial specialisation, it is interesting that epithelial-associated junctional proteins have been found in more ancient organisms and structures resembling epithelia have been described for example in the social amoeba *Dictyostelium discoideum* (Dickinson *et al*, 2011). Cancers arising in epithelial tissues are known as carcinomas and much effort has been devoted to unravelling the molecular programmes that occur during formation and progression of carcinomas. One of the most well-studied features of carcinomas, associated with increased aggressiveness and metastatic spread, is the loss of epithelial integrity and specialisation, called epithelial to mesenchymal transition or EMT.

EMT results in loss of features characteristic of epithelial cells – cell–cell adhesions, polarity and amotility and acquisition of a mesenchymal phenotype – spindled shape, motility and ability to invade. These phenotypic changes are accompanied by a loss of epithelial cell markers such as E-cadherin and increased expression of mesenchymal markers such as N-cadherin, vimentin and fibronectin (Figure 1).

EMT was first described in the early 1980s (Greenburg and Hay, 1982) but was termed as epithelial–mesenchymal transformation. This was later amended to epithelial–mesenchymal transition, to reflect the fact that the changes are reversible by mesenchymal–epithelial transition (MET). EMT is crucial to many of the normal developmental processes of metazoa. For example, in mammals, the early embryo forms as a ball of epithelial-like cells and must undergo EMT to invade and grow in the uterus (a process known as implantation). There has been much debate about how to define EMT; for example, should cells that have developed some mesenchymal features but still retain some epithelial ones be classed as having undergone EMT, or does this represent 'partial' EMT? This remains a subject of continuing controversy, which we will not touch on here. In 2008, three types of EMT were defined at a meeting of experts at Cold Spring Harbor Laboratory (Kalluri and Weinberg, 2009; Zeisberg and Neilson, 2009). Although this was by no means the end of the ongoing discussions about how to define EMT, we believe that it forms a useful basis for discussion for this review and a starting

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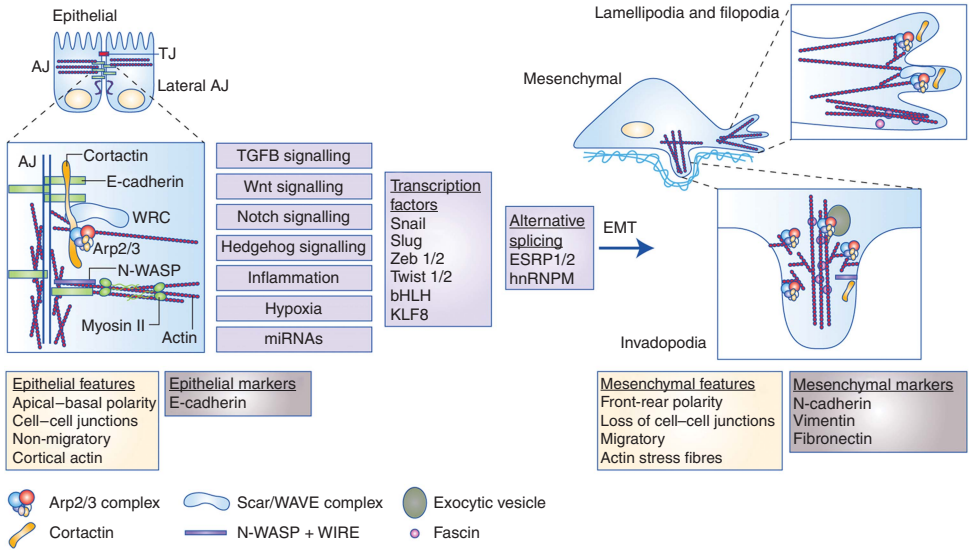

Figure 1. Involvement of actin cytoskeletal reorganisation in EMT. Epithelial cells make tight junctions (TJ, red) that act as a permeability barrier between the outside world and the tissue and adherens juncions (AJ) that provide mechanical stability and strength by connections with the actin cytoskeleton and the transmembrane cadherin proteins. E-cadherins (green) are major scaffold proteins controlling adherens junction integrity and direct links between E-cadherins and actin nucleating proteins, such as WRC (light blue) and Arp2/3 complex (multicoloured) provide a basis for sequestering the actin nucleation machinery when cells are non-motile and also for harnessing actin nucleation to provide actin cytoskeletal scaffolding for tissue integrity. N-WASP (purple) acts with WIRE (not shown) to stabilise and bundle cortical actin filaments (Wu *et al*, 2014). Cortactin (orange) is a scaffold, binding both N-WASP and E-cadherin to recruit ARP2/3 and WRC to adherens junctions. EMT can be driven by a number of signalling pathways (purple boxes, see text for references) that result in the activation of transcriptional programmes and alternative splicing. In mesenchymal cells, E-cadherin is lost and the actin cytoskeleton undergoes a number of changes, resulting in a shift of actin and its regulatory proteins and complexes from the cortex towards the leading edge of migrating cells, where they form lamellipodia. A specialised mesenchymal actin bundling protein, fascin, organises actin filaments at sites of filopodia and is also recruited to invadopodia, the invasive protrusions of cancer cells, along with Arp2/3 complex, cortactin and N-WASP. These changes are accompanied by the expression of mesenchymal markers such as N-cadherin, vimentin and fibronectin and a change in cell polarity (from the apico–basal polarity in epithelial cells to the front–back polarity in mesenchymal cells).

point for gaining a deeper understanding of EMT in development and cancer:

Type 1 EMT: this is the 'normal' process of transition of epithelial cells to a mesenchymal state during implantation and embryonic development, as part of the processes of gastrulation and neural crest formation.

Type 2 EMT: this EMT programme occurs in response to inflammation and is integral to the processes of tissue repair, regeneration and in cases where the inflammatory response is prolonged, fibrosis. This type can also be induced in cancer.

Type 3 EMT: this EMT programme is observed in neoplastic tumour-forming cells as a part of tumour dedifferentiation. Once having undergone EMT, tumour cells are able to invade and migrate to distant sites where they may establish metastases – by definition, conferring properties of malignancy on these cells. This can be accompanied by MET whereby the metastatic tumour nodule once again takes on epithelial characteristics.

For transitions between epithelial and mesenchymal states, cells need to tightly control their motility programmes. Control happens at multiple levels, including gene expression, post-translational modifications and reorganisation of the actin cytoskeleton. Here we discuss EMT during development and in cancer of the pancreas and intestinal tract and we highlight some of the actin cytoskeletal changes that occur during EMT and our emerging understanding of how the cytoskeleton and motility are regulated during these processes.

REPROGRAMMING DURING EMT

All types of EMT are driven by a combination of intrinsic programming of cells and environmental factors, such as signals from the stroma. Two of the most important signalling pathways driving EMT are the transforming growth factor beta (TGFβ) and Wnt pathways (Tam and Weinberg, 2013). Other pathways include receptor tyrosine kinase, Notch and Hedgehog signalling pathways. These signalling pathways trigger a reprogramming of gene expression patterns via various methods, including transcriptional changes, alternative splicing pathways and altered expression of micro-RNA (miR). Transcriptional changes are largely thought to be governed by a handful of so-called EMT Transcription factors or EMT Tfs (Figure 1) that include the Snail superfamily (e.g., Snail, Slug, Zeb1/2, Twist 1/2, bHLH and KLF8; reviewed in Diaz-Lopez *et al*, 2014). These transcription factors repress the epithelial programme of gene expression (e.g., E-cadherin) and enhance mesenchymal expression (e.g., upregulate N-cadherin and vimentin). Alternative splicing occurs in EMT and changes the expression pattern of many proteins via factors such as ESRP1/2 and hnRNPM (Ishii *et al*, 2014; Xu *et al*, 2014). MiRs are small non-coding RNA that interact with mRNA and cause silencing or regulation of transcription (Diaz-Lopez *et al*, 2014). miRs are also major players in the EMT process and are a subject of much research for pancreatic and intestinal cancers, for example, miR-200 and miR34 (Figure 1 and Diaz-Lopez *et al*, 2014). Together these transcriptional and post-transcriptional regulators, driven by signalling pathways from the microenvironment, regulate the various programmes that have been described collectively as EMT, but although there are common threads, different tissues and environmental conexts can trigger quite diverse changes associated with loss of epithelial status and gain of mesenchymal functions.

Downstream of transcriptional changes, alternative splicing and miRNA regulation, many cytoskeletal proteins are altered in their expression, localisation or activity. In addition to the

downregulation of E-cadherin, cells gain the intermediate filament protein vimentin and change their expression patterns of several other adhesion molecules such as integrins and cell surface glycoproteins. They may upregulate other cadherin isoforms, such as N-cadherin. The actin bundling protein fascin is specifically expressed in response to the EMT programme in colorectal and pancreatic cancers (Li *et al*, 2014) but less is known about its role in developmental delamination. Cells undergoing EMT also modulate production of extracellular matrix proteins such as fibronectin (Chen *et al*, 2008; Medici *et al*, 2008). At least in breast cancer, alternative splicing controls proteins such as the actin-binding protein Mena, which switches to an invasion promoting form (Mena-inv) and the membrane receptor CD44 (Goswami *et al*, 2009; Pignatelli *et al*, 2014; Xu *et al*, 2014). The organisation of the actin cytoskeleton is tightly linked to cell–cell junctions in the epithelia and this changes dramatically when cells delaminate and lose their adherens and tight junctions (Figure 1). Many of the actin filament nucleating proteins are not transcriptionally regulated, but are differently localised or regulated. For example, the actin nucleation proteins Scar/WAVE complex, N-WASP, Arp2/3 complex and cortactin localise to cell–cell junctions in epithelial cells, but are released from junctions and redirected to cell-leading edges when cells become mesenchymal (Figure 1). Many of these changes are controlled by the activity of the Rho-family small GTPases, including Rac1 and Cdc42, which have both been implicated in regulating the dynamics of epithelial cell junctions (Woodham and Machesky, 2014).

EMT AND CELL MIGRATION DURING DEVELOPMENT OF THE PANCREAS AND INTESTINAL TRACT

EMT first occurs very early in the development of the intestinal tract. Under the influence of Wnt signalling (Liu *et al*, 1999) and downstream mediators belonging to the TGFβ superfamily (Andersson *et al*, 2007), epiblast cells in the primitive streak of the embryo undergo EMT, migrating internally to produce the mesoderm and endoderm. The mechanisms by which this is orchestrated are reviewed in Chuai *et al* (2012). The epiblast cells that do not undergo EMT remain on the surface, forming the ectoderm (Acloque *et al*, 2009) and subsequently cells of the endoderm form an epithelial tube extending for the length of the embryo. This tube differentiates to form three different sections – the foregut (gives rise to the pharynx, oesophagus and stomach), midgut (gives rise to the small intestine and proximal large intestine) and hindgut (gives rise to the mid and distal large intestine). At midgestation, the endoderm undergoes further differentiation in response to signals from the mesoderm and eventually the intestinal epithelium specialises to form villi and crypts containing specialised cell types.

In contrast to the intestinal lining, which retains its epithelial status from the time of formation of the endoderm, some components of the pancreas and liver require the cells to undergo a further round of EMT and MET. For example, pancreatic bud cells undergo EMT and migrate away from the epithelium to form the endocrine cells of the Islets of Langerhans (Johansson and Grapin-Botton, 2002). E-cadherin expression is repressed in a subset of cells, termed neurogenin3 + -expressing insulin-producing cells, leading to migration and clustering to form islets. The EMT transcription factor Slug (also called Snail2) inversely correlates with E-cadherin expression in the developing pancreas and has been implicated in delamination and migration of the neurogenin3 + endocrine progenitor cells during islet formation (Rukstalis and Habener, 2007).

The Rho-family GTPase Cdc42 and its downstream target N-WASP are key players in pancreatic islet formation, as the expression of constitutively active Cdc42 prevents delamination, disassembly of actin at cell–cell junctions and migration (Kesavan *et al*, 2014). N-WASP depletion can partially rescue this phenotype, suggesting that N-WASP-mediated stabilisation of junctional actin needs to be repressed for the delamination process to complete (Kesavan *et al*, 2009; Kesavan *et al*, 2014). Cdc42 is also implicated in the formation of tubules in the developing pancreas as it has a central role in apical polarisation and thus lumen formation (Kesavan *et al*, 2009). In contrast, Rac1 was implicated in the mobilisation of E-cadherin junctions in the developing islets, as expression of a dominant negative Rac1 prevented migration (Greiner *et al*, 2009).

Under the control of the Rho GTPases, the actin cytoskeleton has an important role in epithelial cell–cell junctions, providing connectivity and strength and serving as a platform for signalling and membrane trafficking. Although next to nothing is known of the specific roles of the actin nucleation proteins in the intestinal tract, the key actin organisers N-WASP, cortactin and Arp2/3 complex have all been implicated in actin dynamics at cell–cell junctions in epithelia in tissue culture systems. Rather than stimulating new actin polymerisation for protrusion and migration, as it does in mesenchymal migrating cells, N-WASP functions together with its binding partner WIRE to stabilise and bundle actin filaments at cell–cell junctions to allow for generation of tension by myosin-II (Wu *et al*, 2014; Figure 1). This junctional tension can regulate whether cells are integrated or excluded from the epithelial monolayers, so is an interesting potential contributor to delamination and extrusion from the epithelium. Ras-transformed cells undergo abnormal extrusion from epithelial tissues (Hogan *et al*, 2009) and this may contribute to cancer cells breaking away from primary tumours and thus gaining access to other tissues in the body. The actin nucleation-promoting protein cortactin is implicated as a major scaffold in epithelial cell junctions, with direct interactions between cortactin, N-WASP and E-cadherin having a role in recruitment of Arp2/3 complex and the Scar/WAVE complex, to promote actin nucleation at adherens junctions (Han *et al*, 2014; Figure 1). In contrast, N-WASP, Scar/WAVE proteins, cortactin and Arp2/3 complex localise to leading edge protrusions of migrating cells, where they contribute to membrane dynamics and protrusion (Figure 1). Clearly, ancient proteins involved in motility, such as WASP-family proteins, have evolved features that allow them to promote epithelial cell organisation when they need to be restricted from nucleating leading edge actin assembly and we propose that gaining a deeper understanding of these features would make a valuable contribution to our understanding of EMT and cancer spread.

EMT IN CANCERS OF THE INTESTINAL TRACT AND PANCREAS

EMT has been widely proposed as a mechanism used by carcinoma cells to regain developmental motile and invasive properties and disseminate throughout the body. Epithelial tumours usually initiate by gradually increasing degrees of dysplasia. For example, colorectal adenocarcinomas usually develop through several benign stages before becoming malignant. First, there is formation of aberrant crypt foci (ACFs, abnormal clusters of crypt cells), some of which may be dysplastic. Continued growth of ACFs results in formation of benign protrusions of epithelium (polyps). Some polyps are simply hyperplastic and generally do not advance beyond this stage. Those containing dysplasia are called adenomas; if they continue to grow and accumulate additional genetic abnormalities, they may progress through low-to-high grade dysplasia and finally to malignant adenocarcinomas (Fearon and Vogelstein, 1990). How well differentiated a cancer is (how much it histologically resembles the normal epithelial tissue) has clinical relevance in terms of prognosis and is expressed in terms of the

'grade' of cancer (Grades 1–3 for colorectal cancer). Likewise, pancreatic ductal adenocarcinoma is thought to arise by gradual increases in the dysplasia, which is graded as Pancreatic Intraepithelial Neoplasia stages 1–3 (Distler *et al*, 2014). The role of EMT transcription factors and EMT in these changes is only partially understood and most of the pancreatic precancerous lesions in a mouse model of PDAC retained E-cadherin junctions even though at later stages they expressed the EMT transcription factor Slug (Li *et al*, 2014). Human intestinal adenomas contain hallmarks of some aspects of EMT (Chen *et al*, 2008).

There is a large body of evidence to suggest that EMT associated changes in gene expression and cell morphology occur in carcinomas and that they contribute to the aggressiveness, invasiveness and spread (Tam and Weinberg, 2013). Studies investigating the prognostic significance of EMT markers in human cancers are summarised in Table 1. Two of the most heavily implicated pathways in cancer EMT are the Wnt and TGFβ signalling pathways. Greater than 80% of all sporadic colorectal carcinomas harbour mutations in the Wnt signalling pathway, such as loss of adenomatous polyposis coli (APC) leading to the constitutive hyperactivation of Wnt signalling. Low E-cadherin correlates with poor survival in multiple clinical studies and is an independent prognostic indicator in at least five studies (Table 1). However, APC loss alone, although it triggers hyperproliferation and benign tumour formation, is insufficient to drive the development of cancer; increasing genetic instability is thought to be another major contributing factor (Bogaert and Prenen, 2014) as is signalling from TGFβ and Wnt signalling from the stroma. EMT is often only apparent at the tumour–stroma interface in colorectal cancers, because there seems to be a threshold of signalling necessary to sustain nuclear β-catenin and to drive invasive behaviour (Brabletz *et al*, 2001). The cells at the leading tumour edges frequently show elevation of Zeb1 transcription factor (reviewed in Schmalhofer *et al* (2009)) and Zeb1 is a Wnt target in colorectal cancer (Sanchez-Tillo *et al*, 2011) that correlates with poor survival (Table 1). Zeb1 promotes EMT changes partly by repression of miR-200 family members ((Burk *et al*, 2008) and Table 1). Several miRs have been implicated as correlating with poor survival in colorectal cancer (Table 1) and low miR-212 is an independent prognostic indicator of poor outcome (Table 1). Other EMT transcription factors, Snail1/2 (Slug) and Twist also have been correlated with poor survival in GI cancers (Table 1) as have hallmarks of EMT such as increased vimentin and fibronectin (Table 1).

In addition to transcriptional changes, EMT in cancer promotes similar changes to the cytoskeleton as developmental EMT, with cell–cell adherens junctions becoming more labile and cell migration increasing. Although we know almost nothing about how this works in pancreatic and intestinal cancers, studies from other cell types might inform future research. For example, in A431 human squamous carcinoma cells, E-cadherin mobility and turnover at junctions increases in invading tumours (Serrels *et al*, 2009). In many cell types, collective invasion, where cells move together in strands, but maintain some junctional contacts with neighbours, can be mediated by the loss of E-cadherin and full or partial replacement with N-cadherin (reviewed recently in Etienne-Manneville (2014)). N-cadherin promotes mobility and has recently been found to treadmill along the adjacent side interfaces between migrating astrocytes to promote collective migration (Peglion *et al*, 2014). N-cadherin has been implicated in EMT changes in colorectal cancer (Hu *et al*, 2014), so these mechanisms may be relevant for tumour invasion. In addition to the breakdown of cell–cell adhesions, proteins such as N-WASP, Scar/WAVE complex, cortactin and Arp2/3 complex mobilise away from junctions and towards the leading edges of cells where they actively induce protrusions that can interact with and remodel the surrounding stroma (Figure 1 and reviewed in McNiven (2013)). Actin polymerisation driven by these protein assemblies drives cell protrusion and migration away from the tumour site. Cells assemble matrix-degrading structures termed invadopodia that contain major actin nucleation proteins and that interface with adhesion and matrix metalloprotease secretion machinery (for recent reviews, see McNiven(2013); Beaty and Condeelis (2014)). The actin bundling protein fascin is also a major target of cancer EMT and is thought to promote invasiveness, migration and metastatic potential in multiple cancer types, including pancreatic (Li *et al*, 2014) and colorectal (Hashimoto *et al*, 2006). Secretion of matrix metalloproteases increases during EMT (Ota *et al*, 2009) and cells gain the ability to migrate through three-dimensional (3D) extracellular matrix and to breach tight barriers such as the basement membranes that surround epithelial organs.

The appearance of tumour buds, or clusters of invaded cells surrounding a tumour, is a feature particularly associated with metastasis and poor prognosis in cancers of the gastrointestinal tract, including colorectal and pancreatic cancer (Park *et al*, 2005; Karamitopoulou *et al*, 2013). These budding cells have many features that support the hypothesis that they have undergone EMT, including decrease or loss of E-cadherin, expression of mesenchymal markers and activation of the Wnt signalling pathway (Lugli *et al*, 2012). A recent study of invasive cancers used 3D reconstruction of serial sections of tumour margins to demonstrate that human pancreatic, lung, breast and colorectal cancers invade almost exclusively as collective strands rather than as individual cells (Bronsert *et al*, 2014). Tumour buds were visualised in 3D reconstructions as strands of cells still attached to the primary tumour that had altered E-cadherin staining, increased expression of Zeb1 and altered polarity features. It would be interesting to know how EMT changes in tumour buds correlate with actin cytoskeletal mobilisation and reorganisation, but this awaits more advanced imaging methods and cancer models.

We have mostly discussed the role of the actin machinery in migration of cells away from the primary tumour, but metastasis involves many steps, including also seeding of escaped tumour cells in distant sites. Two recent studies highlight the actin cytoskeletal and integrin-dependent pathways that contribute to seeding of cancer cells in the lungs and formation of early metastatic nodules (Shibue *et al*, 2012; Shibue *et al*, 2013). The authors identify actin-rich filopod-like protrusions (FLP) that contain integrin and allow cells to attach to matrix and activate their prosurvival and growth pathways via focal adhesion kinase. These FLP structures are enhanced by the actin nucleation formin protein mDia2 and regulated by the small GTPase Rif (Shibue *et al*, 2012). In addition, FLP are enhanced by expression of the integrin:actin linker protein β-parvin (Shibue *et al*, 2013). It is not clear yet whether this pathway is controlled by cancer associated EMT, but expression of the EMT transcription factors Twist or Snail or knockdown of E-cadherin enhanced the FLP pathway, suggesting a potential connection (Shibue *et al*, 2013).

SUMMARY

The formation of epithelia by multicellular organisms has required that cells evolve mechanisms to tightly control protein expression, activation status and localisation. Most epithelial tissues have some plasticity in their differentiation status and can convert between epithelial and mesenchymal if the right signals are given. During cancer, the EMT programme becomes unregulated or misregulated to produce changes that resemble type-1 developmental EMT, but that also have significant differences. Many different signals can provoke EMT-like changes in cancer that lead to breakdown or mobilisation of epithelial junctions and enhance the progression and spread of the cancer. There is a wealth of evidence from the

Table 1. Summary of cancer studies implicating EMT markers in prognosis and outcomes for several epithelial cancers of the gastrointestinal tract

Marker	Authors	Year	Journal	Site	Method	No. of cases	KMC LRT *P*-value	CoxPH HR	HR *P*-value	Outcome	Notes
E-cadherin −											
	Kroepli *et al*	2013	BMC Cancer	Colorectal	TMA IHC	250	0.87	NT		OS	
	Bellovin *et al*	2005	Cancer Res	Colorectal	TMA IHC	557	0.0127	Not shown	NS	OS	
	Knösel *et al*	2012	Int J Colorectal Dis	Colorectal (high grade)	TMA IHC	402	0.083	NT		OS	
	Yun *et al*	2014	Oncology	Colorectal	TMA IHC	409	0.009	1.984 (0.539–7.296)	0.303	OS	
	Yun *et al*	2014	Oncology	Colorectal	TMA IHC	409	0.003	5.098 (1.801–14.430)	0.002	DFS	
	Jie *et al*	2013	Dig Dis Sci	Colorectal	WTB IHC	108	<0.01	NT		OS	
	Shioiri *et al*	2006	Br J Cancer	Colorectal	WTB IHC	138	0.0066	2.249 (1.164–4.343)	0.0158	OS	
	Fujikawa *et al*	2012	J Gastroenterol	Colorectal	qPCR	102	<0.01	Not shown	NS	OS	
	Nitta *et al*	2014	BJC	Bile duct	TMA IHC	117	0.018	2.09 (1.11–4.27)	0.0208	OS	
	Chen *et al*	2014	Tumour Biol	Gallbladder	WTB IHC	93	NT	1.856 (1.034–2.976)	0.026	OS	
	Hou *et al*	2012	Med Oncol	Gastric	WTB IHC	158	<0.001	0.574 (0.371–0.886)	0.012	OS	HR for E-cad positivity
	Kim *et al*	2009	Histopathol	Gastric	TMA IHC	598	0.0006	Not shown	NS	OS	
Summary: 9/10 significant differences in OS, 1/1 significant difference in DFS, 4/8 independent prognostic variable OS, 1/1 independent prognostic variable of DFS											
Snail +											
	Kroepli *et al*	2013	BMC Cancer	Colorectal	TMA IHC	251	0.57	NT		OS	
	Franci *et al*	2009	PLoS One	Colorectal	TMA IHC	162	0.011	NT		OS	
	Kim *et al*	2014	Oncol Rep	Colorectal	qPCR	109	0.014	2.11 (1.03–4.33)	0.041	OS	
	Nitta *et al*	2014	BJC	Bile duct	TMA IHC	117	0.3413	NT		OS	
	Shin *et al*	2012	BMC Cancer	Gastric	TMA IHC	314	0.023	0.590 (0.363–0.958)	0.033	OS	HR for Snail negativity
	Kim *et al*	2009	Histopathol	Gastric	TMA IHC	598	<0.0001	1.31	0.041	OS	
Summary: 4/6 significant differences in OS, 3/3 independent prognostic variable of OS											
Slug +											
	Shioiri *et al*	2006	Br J Cancer	Colorectal	WTB IHC	138	<0.0001	2.212 (1.127–4.342)	0.021	OS	
	Nitta *et al*	2014	BJC	Bile duct	TMA IHC	117	0.6143	NT		OS	
Summary: 1/2 significant difference in OS, 1/1 independent prognostic variable of OS											
Twist +											
Twist 1	Gomez *et al*	2011	PLoS One	Colorectal	qPCR	151	0.001	2.73 (1.5–4.84)	0.001	OS	
	Gomez *et al*	2011	PLoS One	Colorectal	qPCR	151	0.16 (0.02 for Stage 1 only)	1.99 (1.05–3.82)	0.036	DFS	
	Kim *et al*	2014	Oncol Rep	Colorectal	qPCR	109	0.002	2.29 (1.04–5.00)	0.039	OS	
	Nitta *et al*	2014	BJC	Bile duct	TMA IHC	117	0.5203	NT		OS	
	Ru *et al*	2010	Path and Oncol Res	Gastric (Stages 1–3)	TMA IHC	436	<0.05	Not shown	<0.001	OS	
Twist 2	Yu *et al*	2013	World J Gastroenterol	Colorectal	WTB IHC	93	0.015	5.744 (1.347–24.298)	0.018	OS	
	Yu *et al*	2013	World J Gastroenterol	Colorectal	WTB IHC	93	0.012	3.264 (1.455–7.375)	0.004	DFS	
Summary: 4/5 significant differences in OS, 2/2 signficant difference in DFS, 4/4 independent prognostic variable OS, 2/2 significance as independent prognostic variable of DFS											
Zeb +											
Zeb 1	Liu *et al*	2012	Cancer Sci	Colorectal	WTB IHC	203	<0.05	NT		OS	
	Zheng *et al*	2013	Oncol Lett	Colorectal	qPCR	92	0.01	2.237 (1.008–4.968)	0.048	OS	
	Nitta *et al*	2014	BJC	Bile duct	TMA IHC	117	0.379	NT		OS	
	Bronsert *et al*	2014	Surgery	Pancreas (Tumour)	WTB IHC	112	0.043	Not shown	NS	OS	
	Bronsert *et al*	2014	Surgery	Pancreas (Stroma)	WTB IHC	112	0.032	1.772 (1.033–3.041)	0.038	OS	
Zeb 2	Kahlert	2011	Cancer Sci	Colorectal (invasive front)	IHC	175	<0.0001	2.48 (1.16–5.27)	0.02	CSS	
	Nitta *et al*	2014	BJC	Bile duct	TMA IHC	117	0.938	NT		OS	
	Dai *et al*	2012	Dig Dis Sci	Gastric	WTB IHC	76	<0.05	NS		OS	
Summary: 5/7 significant differences in OS, 1/1 signficant difference in CSS, 2/3 independent prognostic variable of OS, 1/1 showed significance as independent prognostic variable of CSS											

Table 1. (Continued)

Marker	Authors	Year	Journal	Site	Method	No. of cases	KMC LRT *P*-value	CoxPH HR	HR *P*-value	Outcome	Notes
Vimentin +											
	Yun *et al*	2014	Oncology	Colorectal	TMA IHC	409	NT	0.769 (0.419–1.413)	0.398	OS	
	Nitta *et al*	2014	BJC	Bile duct	TMA IHC	117	0.0193	1.21 (0.61–2.25)	0.5662	OS	
	Chen *et al*	2014	Tumour Biol	Gallbladder	WTB IHC	93	NT	1.645 (0.956–2.756)	0.043	OS	
	Kim *et al*	2009	Histopathol	Gastric	TMA IHC	598	0.0008	Not shown	NS	OS	
	Hou *et al*	2012	Med Oncol	Gastric	WTB IHC	158	0.029	1.444 (0.910–2.291)	0.119	OS	
	Otsuki *et al*	2011	Oncol Rep	Gastric	qPCR	106	0.019	2.1 (1–4.4)	0.036	DFS	
Summary: 3/3 significant differences in OS, 1/1 signficant difference in DFS, 1/5 independent prognostic variable of OS, 1/1 independent prognostic variable of DFS											
Fibronectin +											
	Yun *et al*	2014	Oncology	Colorectal	TMA IHC	409	NT	0.802 (0.437–1.474)	0.478	OS	
	Nitta *et al*	2014	BJC	Bile duct	TMA IHC	117	0.0092	1.08 (0.64–1.79)	0.9093	OS	
Summary: 1/1 significant difference in OS, 0/2 signficance as independent variable of OS											
alpha-SMA +											
	Yun *et al*	2014	Oncology	Colorectal	TMA IHC	409	NT	0.997 (0.611–1.627)	0.991	OS	
	Nitta *et al*	2014	BJC	Bile duct	TMA IHC	117	0.5216	NT		OS	
Summary: 0/1 significant difference in OS, 0/1 independent variable of OS											
N-cadherin +											
	Jie *et al*	2013	Dig Dis Sci	Colorectal	WTB IHC	108	0.41	NT		OS	
	Nitta *et al*	2014	BJC	Bile duct	TMA IHC	117	0.0004	2.53 (1.36–4.54)	0.0038	OS	
	Kim *et al*	2009	Histopathol	Gastric	TMA IHC	598	0.002	Not shown	NS	OS	
Summary: 2/3 significant differences in OS, 1/1 independent variable of OS											
TGF-Beta +											
	Calon *et al*	2012	Cancer Cell	Colorectal	qPCR	335	Not shown	100	<0.0001	DFS	
Summary: 1/1 signficance as independent variable of OS											
miR											
miR-132 (low)	Zheng *et al*	2014	World J Gastroenterol	Colorectal	qPCR	62	<0.001	NT		DFS	
miRNA-19b (high)	Kahlert	2011	Cancer Sci	Colorectal liver mets	qPCR	30	0.04	NT		OS	
miRNA-19b (high)	Kahlert	2011	Cancer Sci	Colorectal liver mets	qPCR	30	0.002	NT		DFS	
miR-194 (high)	Kahlert	2011	Cancer Sci	Colorectal liver mets	qPCR	30	0.003	NT		OS	
miR-194 (high)	Kahlert	2011	Cancer Sci	Colorectal liver mets	qPCR	30	0.008	NT		DFS	
miR-212 (low)	Meng *et al*	2013	Gastroenterology	Colorectal	qPCR	180	0.0015	0.403 (0.195–0.829)	0.014	OS	HR for high miR-212
miR-212 (low)	Meng *et al*	2013	Gastroenterology	Colorectal	qPCR	180	0.0045	NT		DFS	
miR-30a (low)	Liu *et al*	2014	Febs Letters	Hepatocellular	qPCR	63	0.015	3.2 (1.5–6.8)	0.002	DFS	
Summary: 3/3 significant differences in OS, 5/5 significant difference in DFS, 1/1 signficance as independent variable of OS, 1/1 significance as independent variable of DFS											
Combination ("mesenchymal phenotype")											
Vim: E-cad ratio >1.24	Mashita *et al*	2014	J Surg Oncol	Colorectal	qPCR	150	0.0085	1.48 (0.47–4.35)	0.485	DFS	
Snail1 +, Vimentin +, E-cad −, CD44 +	Ryu *et al*	2012	Hum Pathol	Gastric	TMA IHC	276	<0.001	2.072 (1.077–3.986)	0.29	DFS	
Snail1 +, Vimentin +, E-cad -, CD44 +	Ryu *et al*	2012	Hum Pathol	Gastric	TMA IHC	276	<0.001	1.930 (0.993–3.752)	1.052	OS	
Low E-cad, vimentin +	Lahat *et al*	2014	Ann Surg Oncol	Pancreas (IPMN)	WTB IHC	33	0.007	1.93 (1.4–3.77)	0.05	OS	
Twist +, Bmi-1 +	Ishikawa *et al*	2014	J Gastroenterol Hepatol	Pancreatic (IPMN)	WTB IHC	35	<0.05	NT		DFS	
Summary: 2/2 significant differences in OS, 3/3 significant difference in DFS, 1/2 signficance as independent variable of OS, 0/2 significance as independent variable of DFS											

Abbreviations: CoxPH = Cox proportional hazards multivariate analysis; CSS = cancer-specific survival; DFS = disease-free survival; HR = hazard ratio; IHC = immunohistochemistry; IPMN = intraductal papillary mucinous neoplasm; KMC LRT = Kaplan–Meier survival curve log-rank test; NS = not significant; NT = not tested; OS = overall survival; qPCR = quantitative PCR; TMA = tissue microarray; WTB = whole tissue blocks. Recent studies showing the usefulness of various markers of EMT, such as transcription factors, cytoskeletal markers and micro-RNAs are summarised.

clinical literature suggesting a positive correlation between EMT signalling and transcriptional changes and poor outcome in many cancers, including those of the pancreas and intestinal tract.

Very little is known about how the motility machinery reorganises during EMT. Although the loss of E-cadherin junctions is the most prominent feature of most EMT transitions, many other changes occur and the actin nucleation-promoting proteins such as N-WASP, Scar/WAVE and cortactin have specific roles both in epithelial and mesenchymal cells. Rho-family GTPases participate in regulation of the actin cytoskeleton in both epithelial and mesenchymal cells and seem to have important roles in developmental and cancer-related EMT.

Cancer EMT is clearly very different from developmental EMT, but parallels exist and EMT-related changes in cancer correlate strongly with progression and poor outcome. Cancer EMT can be partial and both solid tumours and circulating tumour cells may co-express epithelial and mesenchymal markers (Armstrong *et al*, 2011). Furthermore, the mesenchymal status is not sufficient in all cases to confer metastasis, as there are some benign tumours (which by definition, do not usually metastasise) that typically show aggressive local invasion, for example, giant cell tumour of the bone (Fletcher *et al*, 2002) and ameloblastoma (Barnes, 2005). The importance of EMT in cancer has been challenged (Tarin *et al*, 2005) and it appears that many tumours that histologically are 'epithelial' can be aggressively metastatic. Many questions remain about which aspects of EMT promote metastatic dissemination and how cancers hijack developmental EMT to progress. Likewise, the precise regulation of key actin motility proteins during EMT and MET is only beginning to be understood and may provide insight that will be clinically useful.

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