25. Heldin, C. H., Eriksson, U. & Ostman, A. New members of the platelet-derived growth factor family of mitogens. *Arch. Biochem. Biophys*. **398**, 284–290 (2002).
26. Rao, G. N., Delafontaine, P. & Runge, M. S. Thrombin stimulates phosphorylation of insulin-like growth factor-1 receptor, insulin receptor substrate-1, and phospholipase C-γ-1 in rat aortic smooth muscle cells. *J. Biol. Chem*. **270**, 27871–27875 (1995).
27. Lee, F. S. & Chao, M. V. Activation of Trk neurotrophin receptors in the absence of neurotrophins. *Proc. Natl Acad. Sci. USA*. **98**, 3555–3560 (2001).
28. Peng, H. *et al.* Integrative nuclear FGFR1 signaling (INFS) pathway mediates activation of the tyrosine hydroxylase gene by angiotensin II, depolarization and protein kinase C. *J. Neurochem*. **81**, 506–524 (2002).
29. Östman, A. & Böhmer, F. D. Regulation of receptor tyrosine kinase signaling by protein tyrosine phosphatases. *Trends Cell. Biol*. **11**, 258–266 (2001).
30. Meng, T. C., Fukada, T. & Tonks, N. K. Reversible oxidation and inactivation of protein tyrosine phosphatases *in vivo*. *Mol. Cell* **9**, 387–399 (2002).
31. Sundaresan, M., Yu, Z. X., Ferrans, V. J., Irani, K. & Finkel, T. Requirement for generation of H_2O_2 for platelet-derived growth factor signal transduction. *Science* **270**, 296–299 (1995).
32. Rhee, S. G., Bae, Y. S., Lee, S. R. & Kwon, J. Hydrogen peroxide: a key messenger that modulates protein phosphorylation through cysteine oxidation. *Sci. STKE* 2000, pe1 (2000).
33. Chen, Q., Olashaw, N. & Wu, J. Participation of reactive oxygen species in the lysophosphatidic acid-stimulated mitogen-activated protein kinase kinase activation pathway. *J. Biol. Chem*. **270**, 28499–28502 (1995).
34. Patterson, C. *et al.* Stimulation of a vascular smooth muscle cell NAD(P)H oxidase by thrombin. Evidence that p47(phox) may participate in forming this oxidase *in vitro* and *in vivo*. *J. Biol. Chem*. **274**, 19814–19822 (1999).
35. Liebmann, C. Regulation of MAP kinase activity by peptide receptor signalling pathway: paradigms of multiplicity. *Cell. Signal*. **13**, 777–785 (2001).
36. Lopez-Ilasaca, M., Crespo, P., Pellici, P. G., Gutkind, J. S. & Wetzker, R. Linkage of G protein-coupled receptors to the MAPK signaling pathway through PI 3-kinase γ. *Science* **275**, 394–397 (1997).
37. Schmitt, J. M. & Stork, P. J. β2-adrenergic receptor activates extracellular signal-regulated kinases (ERKs) via the small G protein rap1 and the serine/threonine kinase B-Raf. *J. Biol. Chem*. **275**, 25342–25350 (2000).
38. Andreev, J. *et al.* Src and Pyk2 mediate G-protein-coupled receptor activation of epidermal growth factor receptor (EGFR) but are not required for coupling to the mitogen-activated protein (MAP) kinase signaling cascade. *J. Biol. Chem*. **276**, 20130–20135 (2001).
39. Yart, A. *et al.* A function for phosphoinositide 3-kinase β lipid products in coupling βγ to Ras activation in response to lysophosphatidic acid. *J. Biol. Chem*. **277**, 21167–21178 (2002).
40. Della Rocca, G. J., Maudsley, S., Daaka, Y., Lefkowitz, R. J. & Luttrell, L. M. Pleiotropic coupling of G protein-coupled receptors to the mitogen-activated protein kinase cascade. Role of focal adhesions and receptor tyrosine kinases. *J. Biol. Chem*. **274**, 13978–13984 (1999).
41. Adomeit, A. *et al.* Bradykinin B(2) receptor-mediated mitogen-activated protein kinase activation in COS-7 cells requires dual signaling via both protein kinase C pathway and epidermal growth factor receptor transactivation. *Mol. Cell. Biol.* **19**, 5289–5297 (1999).
42. Sabri, A., Short, J., Guo, J. & Steinberg, S. F. Protease-activated receptor-1-mediated DNA synthesis in cardiac fibroblast is via epidermal growth factor receptor transactivation: distinct PAR-1 signaling pathways in cardiac fibroblasts and cardiomyocytes. *Circ. Res*. **91**, 532–539 (2002).
43. Brummer, T., Shaw, P. E., Reth, M. & Misawa, Y. Inducible gene deletion reveals different roles for B-Raf and Raf-1 in B-cell antigen receptor signalling. *EMBO J*. **21**, 5611–5622 (2002).
44. Hanke, S., Nürnberg, B., Groll, D. H. & Liebmann, C. Cross talk between β-adrenergic and bradykinin B(2) receptors results in cooperative regulation of cyclic AMP accumulation and mitogen-activated protein kinase activity. *Mol. Cell. Biol*. **21**, 8452–8460 (2001).
45. Sabri, A. *et al.* Mechanisms of protease-activated receptor-4 actions in cardiomyocytes: role of Src tyrosine kinase. *J. Biol. Chem*. **278**, 11714–11720 (2003).
46. Siekhaus, D. E. & Drubin, D. G. Spontaneous receptor-independent heterotrimeric G-protein signalling in an RGS mutant. *Nature Cell Biol*. **5**, 231–235 (2003).
47. Wennström, S. & Downward, J. Role of phosphoinositide 3-kinase in activation of ras and mitogen-activated protein kinase by epidermal growth factor. *Mol. Cell Biol*. **19**, 4279–4288 (1999).
48. Rubio, I. & Wetzker, R. A permissive function of phosphoinositide 3-kinase in Ras activation mediated by inhibition of GTPase-activating proteins. *Curr. Biol*. **10**, 1225–1228 (2000).
49. Piiper, A. *et al.* Cholecystokinin stimulates extracellular signal-regulated kinase through activation of the epidermal growth factor receptor, Yes, and protein kinase C. Signal amplification at the level of Raf by activation of protein kinase Cε. *J. Biol. Chem*. **278**, 7065–7072 (2003).
50. Thuringer, D., Maulon, L. & Frelin, C. Rapid transactivation of the vascular endothelial growth factor receptor KDR/Flk-1 by the bradykinin B2 receptor contributes to endothelial nitric-oxide synthase activation in cardiac capillary endothelial cells. *J. Biol. Chem*. **277**, 2028–2032 (2002).
51. Gilmore, A. P. *et al.* Activation of BAD by therapeutic inhibition of epidermal growth factor receptor and transactivation by insulin-like growth factor receptor. *J. Biol. Chem*. **277**, 27643–27650 (2002).
52. Zwick, E. *et al.* Critical role of calcium-dependent epidermal growth factor receptor transactivation in PC12 cell membrane depolarization and bradykinin signaling. *J. Biol. Chem*. **272**, 24767–24770 (1997).
53. Moro, L. *et al.* Integrins induce activation of EGF receptor: role in MAP kinase induction and adhesion-dependent cell survival. *EMBO J*. **17**, 6622–6632 (1998).
54. Chen, N. *et al.* Transactivation of the epidermal growth factor receptor is involved in 12-*O*-tetradecanoylphorbol-13-acetate-induced signal transduction. *J. Biol. Chem*. **276**, 46722–46728 (2001).
55. Delafontaine, P., Anwar, A., Lou, H. & Ku, L. G-protein coupled and tyrosine kinase receptors: evidence that activation of the insulin-like growth factor I receptor is required for thrombin-induced mitogenesis of rat aortic smooth muscle cells. *J. Clin. Invest*. **97**, 139–145 (1996).
56. Herrlich, A. *et al.* Ligand-independent activation of platelet-derived growth factor receptor is a necessary intermediate in lysophosphatidic acid-stimulated mitogenic activity in L cells. *Proc. Natl Acad. Sci. USA* **95**, 8985–8990 (1998).
57. Linseman, D. A., Benjamin, C. W. & Jones, D. A. Convergence of angiotensin II and platelet-derived growth factor receptor signaling cascades in vascular smooth muscle cells. *J. Biol. Chem.* **270**, 12563–12568 (1995).
58. Zhang, Y., Jiang, J., Black, R. A., Baumann, G. & Frank, S. J. Tumor necrosis factor-α converting enzyme (TACE) is a growth hormone binding protein (GHBP) sheddase: the metalloprotease TACE/ADAM-17 is critical for (PMA-induced) GH receptor proteolysis and GHBP generation. *Endocrinology* **141**, 4342–4348 (2000).
59. Yan, Y., Shirakabe, K. & Werb, Z. The metalloprotease Kuzbanian (ADAM10) mediates the transactivation of EGF receptor by G protein-coupled receptors. *J. Cell. Biol*. **158**, 221–226 (2002).
60. Lemjabbar, H. & Basbaum, C. Platelet-activating factor receptor and ADAM10 mediate responses to *Staphylococcus aureus* in epithelial cells. *Nature Med*. **8**, 41–46 (2002).
61. Gschwind, A. Hart, S. Fischer, O. M. & Ullrich, A. TACE cleavage of proamphiregulin regulates GPCR-induced proliferation and motility of cancer cells. *EMBO J*. **22**, 2411–2421 (2003).
62. Salmeen *et al.* Redox regulation of protein tyrosine phosphatase 1B involves a sulphenyl-amide intermediate. *Nature* **423**, 769–773 (2003).
63. van Montfort, R. L., Congreve, M., Tisi, D., Carr, R. & Jhoti, H. Oxidation state of the active-site cysteine in protein tyrosine phosphatase 1B. *Nature* **423**, 773–777 (2003).

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OPINION

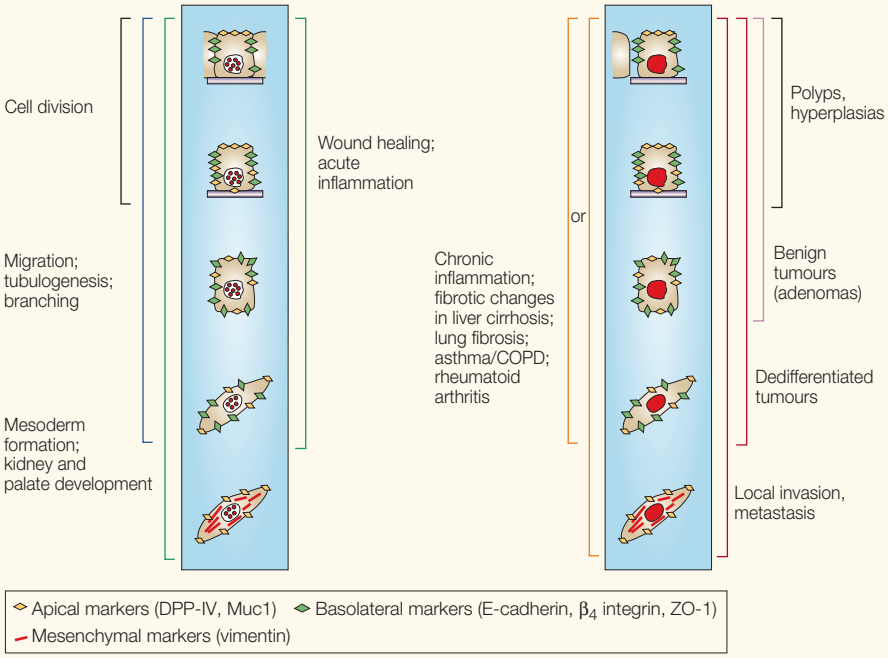
Diverse cellular and molecular mechanisms contribute to epithelial plasticity and metastasis

Stefan Grünert, Martin Jechlinger and Hartmut Beug

In contrast to the aberrant control of proliferation, apoptosis, angiogenesis and lifespan, the cellular mechanisms that cause local invasion and metastasis of tumour cells are still poorly understood. New experimental approaches have identified different types of epithelial-plasticity changes in tumour cells towards fibroblastoid phenotypes as crucial events that occur during metastasis, and many molecules and signalling pathways cooperate to trigger these processes.

The mechanisms that oncogenes or tumour-suppressor genes use during malignant transformation are increasingly well understood[1,2]. However, the emphasis that surrounds crucial events during tumour progression is changing, as many molecular and epigenetic changes seem to cooperate in altering tumour-cell behaviour. These crucial events include altered responses/interactions of tumour cells to humoral and cellular environmental cues, such as paracrine and autocrine factors, tumour stroma and immune cells[2,3]. However, molecular understanding of these

Box 1 | **Normal and pathological epithelial-plasticity phenotypes**



This simple scheme depicts the speculative idea that loss of epithelial polarity and acquisition of a fibroblastoid, mesenchymal phenotype occurs to varying degrees in normal cells (dotted nuclei) in many processes during development and tissue repair in adults (left panel), as well as in genetically altered cells (full nuclei) during numerous pathological processes (right panel). The cell cartoons depict (top to bottom): fully polarized epithelial cells; partial loss of epithelial polarity; complete loss of epithelial polarity; migration/scattering (without sustained gene-expression changes); and epithelial–mesenchymal transition (EMT, including loss of epithelial and gain of mesenchymal markers). COPD, chronic obstructive pulmonary disease; DPP-IV, dipeptidylpeptidase IV; ZO-1, zona occludens 1.

complex events has been impeded by the lack of communication between mechanistic research on model systems[2] and the inherently more descriptive studies on classification and diagnosis of human cancer[4]. This is being overcome by the recognition that tumour progression/dedifferentiation might resemble complex cellular processes that also occur during early development, tissue remodelling and inflammatory processes[5].

Here, we will mainly focus on epithelial–mesenchymal transition (EMT) and the signalling pathways that regulate this process. EMT was initially defined as a process during which epithelial cells: first, acquire a fibroblastoid, invasive phenotype; second, downregulate epithelial-specific proteins (for example, tight- and adherens-junction proteins) and induce various mesenchymal proteins (for example, vimentin; see BOX 1 and BOX 2c); and third, digest and migrate through the extracellular matrix (ECM)[4,6]. The recent, broader use of the term EMT, however — in an attempt to cover any one of the diverse changes in epithelial cells towards fibroblastoid morphology — has considerably confused the field. Our focus on Ras plus transforming growth factor-β (TGF-β) to regulate EMT and metastasis should not belittle the potential importance of other mechanisms — for example, those that involve scatter factor (SF; also termed hepatocyte growth factor, HGF) or fibroblast growth factor (FGF) (see REF. 4 and references therein).

Epithelial plasticity and EMT *in vivo*

Many processes during embryonic development and in adult organisms might cause epithelial cells to lose their polarized phenotype. Transient loss of polarity, accompanied by redistribution of tight- and adherens-junction markers (for example, E-cadherin or zona occludens 1 (ZO-1)) and migration/spreading of cells or cell groups, occur in many developmental processes. These include tubulogenesis and branching in the mammary gland, or tissue reorganization during wound healing. Several other normal processes — such as mesoderm formation during gastrulation, palate fusion and emigration of neural-crest cells from the neural tube — involve EMT, including transcriptional loss of epithelial markers (E-cadherin) plus induction of vimentin[4,7–10] (BOX 1).

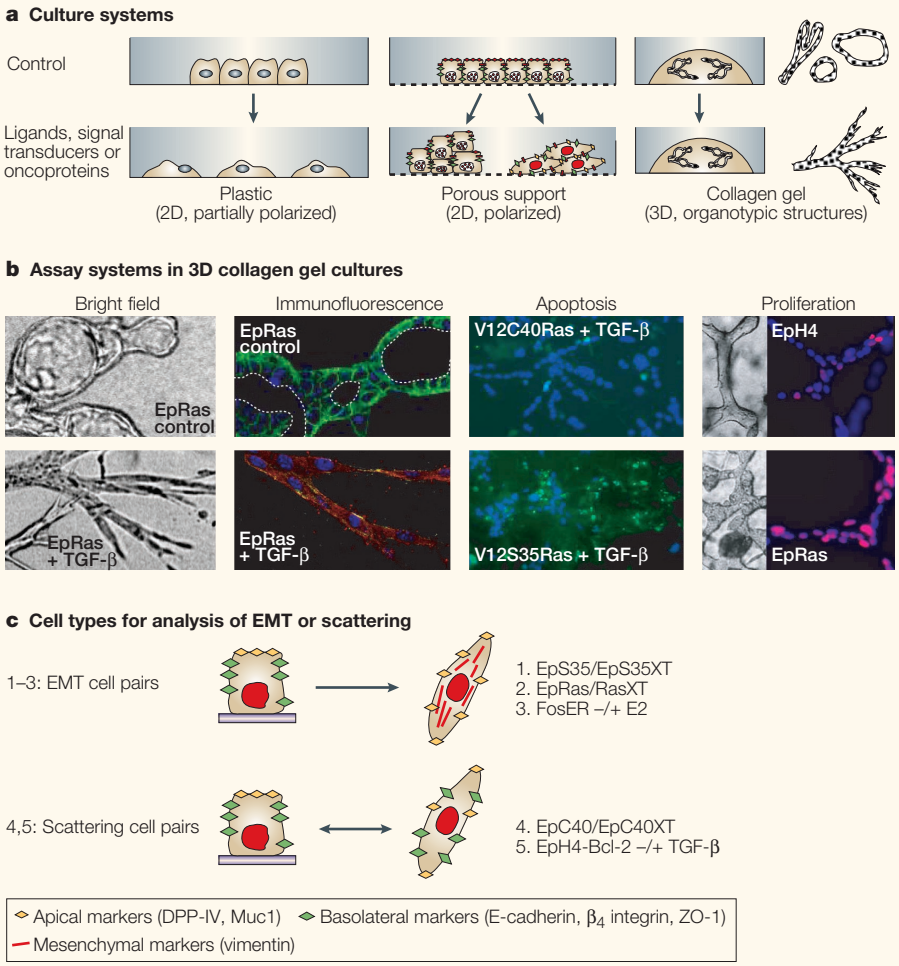
Significantly, a similar variety of epithelial-plasticity changes also occur during pathological processes[11,12] (BOX 1). Chronic inflammation involves the loss of epithelial polarity, altered deposition of ECM proteins, and migratory processes, and contributes to diseases such as lung and kidney fibrosis, chronic obstructive pulmonary disease and chronic asthma, which perhaps also involve the dedifferentiation of epithelial cells into myofibroblasts[13].

Progression of carcinoma cells in dedifferentiated, metastatic tumours also involves many epithelial-plasticity changes[4,14,15] (BOX 1). Often, processes that resemble chronic inflammation accompany tumour progression[5]. Epithelial cells from benign tumours (adenomas) show altered epithelial polarity, whereas progression towards more dedifferentiated, malignant, human tumours involves changes towards migratory, fibroblastoid cell types, including EMT[14,15]. The extent to which this takes place has been hard to determine — in some tumours, EMT is transient, occurs only at invasion fronts of metastasizing primary carcinomas and can be reversed after the establishment of metastasis[4,16]. Furthermore, standard histology of human tumours fails to detect mesenchymal cells that are formed during EMT, as they are indistinguishable from fibroblastoid, tumour stroma cells. However, by co-staining for basal cytokeratins (which are still present in dedifferentiated epithelial cells, but absent in fibroblastic cells) and EMT markers such as vimentin, EMT has been shown to occur frequently[17].

EMT in culture: diverse phenotypes

Signal-transduction pathways that modulate epithelial plasticity have been analysed in culture using different epithelial cell lines. In these studies, the term 'EMT' has been used in a rather loose fashion to encompass a much more diverse set of epithelial-plasticity phenotypes — thereby creating confusion in the field. Many of these experiments have been carried out in standard, two-dimensional (2D) cultures in plastic dishes (BOX 2a, left panel). This impairs epithelial polarization, as nutrients and growth factors cannot pass through the tight junctions of a fully polarized monolayer and so fail to reach their basolaterally-located receptors that face the plastic surface. This problem has been circumvented by culturing the cells on porous supports (BOX 2a, middle panel), which allows the factors access to the basolateral domain, or in three-dimensional (3D) collagen-gel

Box 2 | **Epithelial plasticity: cell culture systems, assay methods and cell pairs used**



The schematics in (**a**) illustrate the different cell culture systems — plastic, porous supports (filters) and three-dimensional (3D) collagen gels — that are used to analyse epithelial-plasticity phenotypes before (top) or after (bottom) induction by ligands, signal transducers or oncoproteins. For 3D cultures, both the culture design and the organotypic structures obtained are shown. The dotted nuclei represent normal cells, whereas the full nuclei are indicative of genetically altered cells.

The images in (**b**) show the cellular properties that can be analysed in 3D collagen cultures. EpRas cells before (top) or after (bottom) epithelial–mesenchymal transition (EMT) in 3D collagen cultures are shown in bright field (far-left panels) or after *in situ* immunofluorescence staining for E-cadherin (green) plus vimentin (red) and analysis by confocal microscopy (middle-left panels)[18]. Apoptosis-resistant V12C40Ras cells (hyperactivated phosphatidylinositol 3-kinase (PI3K) pathway, top) and apoptosis-sensitive V12S35Ras cells (hyperactivated extracellular-signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) pathway, bottom) are shown as an example to measure apoptosis by TUNEL (TdT-mediated dUTP nick-end labelling) staining (middle-right, green). Hyperproliferation of EpRas cells (far-right panel, bottom) compared with control cells (EpH4, far-right panel, top) is shown both in bright field (far-right panel, left-hand section) and after bromodeoxyuridine BrdU-staining (pink; far-right panel, right-hand section) to show proliferating cells. Part **b** is reproduced with permission from REF. 18 © (2002) The Rockefeller University Press and REF. 21 © (2002) Macmillan Magazines Ltd.

The simple cartoons in (**c**) illustrate the morphology and epithelial/mesenchymal marker redistribution or expression that is typical for EMT and scattering. The different cell pairs used for cellular and molecular analysis (that is, expression profiling) of EMT and scattering (driven by different oncogenes and/or growth factors) are also listed. DPP-IV, dipeptidylpeptidase IV; EpS35, V12S35Ras cells; EpC40, V12C40Ras cells; FosER, EpH4 cells expressing an oestrogen (E2)-activatable oestrogen receptor–c-Fos fusion protein; XT, Ras or Ras mutant cells recultivated from tumours or collagen gels; ZO-1, zona occludens 1.

cultures, in which fully polarized cells form organotypic structures (BOX 2a, right panel; BOX 2b).

In addition, cells have been exposed to factors or inducible oncogenes using very different experimental regimes. Obviously, this diversity in several parameters could account for the inconsistent or even conflicting results that have been obtained. FIGURE 1 summarizes these findings, and groups experiments according to the type of epithelial-plasticity change observed, the cell culture systems used (BOX 2a), and the signalling pathway or pathways involved.

Scattering versus EMT. Most studies have used cells cultured on plastic and exposed to growth factors, inhibitors or inducible oncogenes, for 48 hours or less. Under these conditions, cells became migratory and fibroblastoid in shape and lost epithelial polarity, with a concomitant redistribution/reduced expression of epithelial markers (E-cadherin); but, they failed to turn on a mesenchymal gene-expression programme[24–27]. This phenotype is induced by SF/HGF, TGF-β, FGFs and epidermal growth factor (EGF)/transforming growth factor-α and — dependent on the cell type — involves the extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) pathways, as well as the small GTPases Rac and Rho. As this phenotype was first observed with scatter factor, we refer to it as 'scattering' (for more information and references, see FIG. 1c, REF. 18 and ONLINE TABLE 1).

In contrast to scattering, full EMT is completed only after >4–6 days of exposure to several signals, and occurs only in certain cell types, some of which require 3D culture conditions to rapidly and synchronously undergo EMT. As some cells that are capable of EMT undergo scattering after short-term factor treatment, one crucial parameter for inducing scattering or EMT might be acute versus chronic signal exposure (see FIG. 1d, REF. 18 and ONLINE TABLE 1).

Ras in cell plasticity. Expression of oncogenic Ras in various epithelial cell types and culture

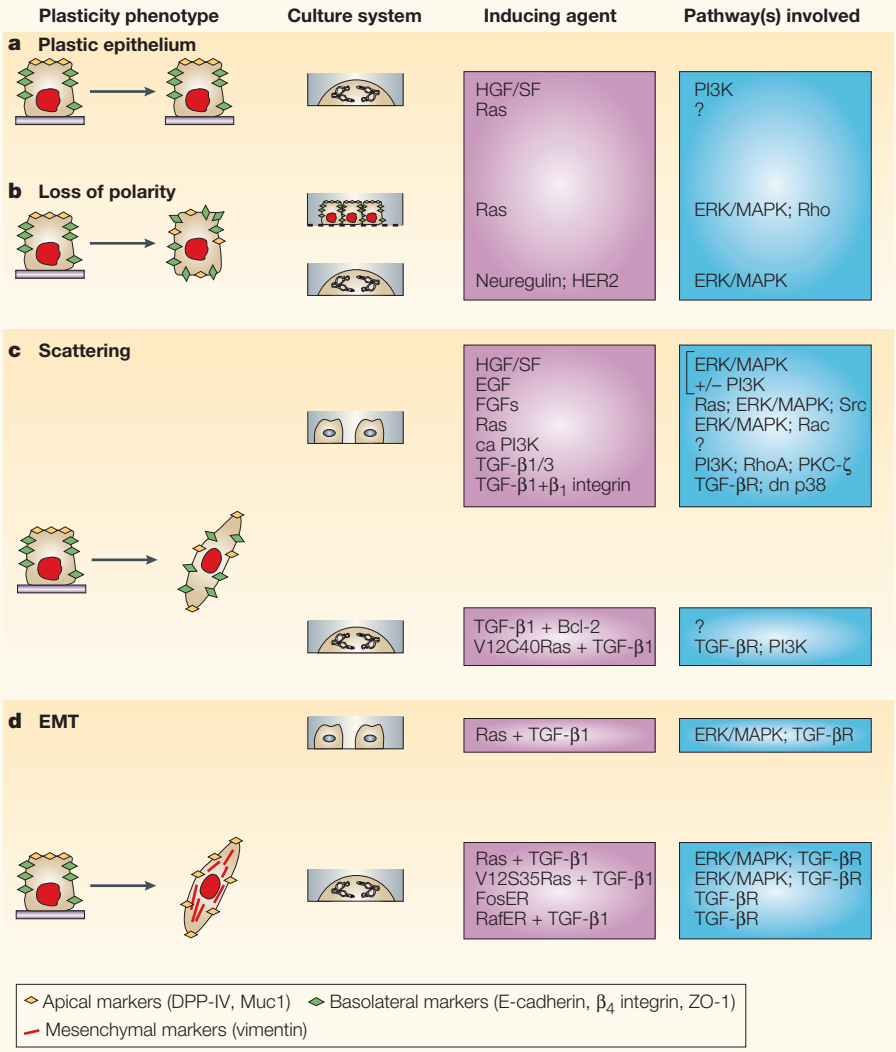


Figure 1 | **Diversity of epithelial phenotypes**. This schematic shows the diversity of phenotypes that were induced by growth factors, signal transducers and oncoproteins. The phenotypes obtained were grouped: first, according to four different epithelial-plasticity phenotypes induced by respective agents (indicated by the cell schemes outlined in BOX 1); second, according to the cell-culture system used (indicated by cartoons for plastic, filters and 3D culture: see BOX 2a); third, according to the inducing agents that are involved; and fourth, according to the signalling pathways that are involved. For details of the various cell systems and signal-transduction tools used, and for respective references, see ONLINE TABLE 1. ca, constitutively active; dn, dominant-negative; DPP-IV, dipeptidylpeptidase IV; EGF, epidermal growth factor; ER, oestrogen receptor; ERK, extracellular signal-regulated kinase; FGF, fibroblast growth factor; SF/HGF, scatter factor/hepatocyte growth factor; MAPK, mitogen-activated protein kinase; PKC-ζ, protein kinase C ζ; PI3K, phosphatidylinositol 3-kinase; R, receptor; TGF-β, transforming growth factor-β; ZO-1, zona occludens 1.

conditions has also yielded partially conflicting results. In cells cultured on plastic, either H-Ras or constitutively active ERK/MAPK was sufficient to cause scattering (FIG. 1c), whereas similar cells grown on filters lost only apical, but not basolateral, polarity. Apart from subline variability, the different culture conditions might account for various discrepancies. For example, Ras transformation might destabilize — rather than abolish — tight and adherens junctions, which could lead to a scattering phenotype on plastic, whereas epithelial polarity would be partially or fully maintained on filters or 3D collagen cultures, respectively (see FIG. 1a–c and ONLINE TABLE 1).

Plasticity in 3D culture

EpH4 cells — an epithelial cell line spontaneously immortalized from mid-pregnant mouse mammary glands — form organotypic, tubular structures in 3D serum-free collagen I gels[19,20] (FIG. 2a). Recently, this 3D culture system has been adapted to analyse cell morphology and behaviour; epithelial and mesenchymal marker gene and protein expression; apoptosis; and cell proliferation[18,21] (BOX 2b). This has allowed the analysis of EpH4-cell responses to different oncogenes and/or various growth/differentiation factors in a more physiological way than is possible in conventional 2D cultures (see REFS 18,20 and references therein). According to morphology, polar or non-polar marker localization and epithelial versus mesenchymal marker expression, at least four different types of epithelial plasticity could be evoked in EpH4 cells by different signals or signal combinations.

Plastic epithelium. Both EpH4 cells and EpRas cells (the counterparts of EpH4 cells that express oncogenic Ras) form tubular, branched structures of fully polarized cells, but EpRas cells grow faster (BOX 2b) and mainly form alveolar structures with larger lumina (FIG. 2a). We refer to this EpRas phenotype as 'plastic epithelium', to highlight the increased epithelial plasticity and migratory capacity of the EpRas cells, which is also observed in Ras-transformed kidney- and liver-derived cell systems[20,22,23] (FIG. 1a).

Polarity loss. Complete loss of epithelial polarity in the absence of induced migration — as indicated by non-invasive structures with collapsed lumina, redistribution of apical and basal markers, and partial disruption of adherens-junction integrity (FIG. 1b) — can be selectively induced in EpH4 cells by an inducible fusion protein containing the Jun transcription factor[19] (FIG. 2b). This indicates that loss of epithelial polarity is not necessarily a consequence of induced migration or acquisition of a fibroblastoid phenotype.

Scattering and complete EMT. In EpRas cells, two more pronounced phenotypic changes — scattering and EMT — are induced by different signals. Significantly, both phenotypes similarly show a loss of epithelial polarity, a fibroblastoid morphology and enhanced motility, but can be distinguished by marker gene analysis and reversibility studies (FIG. 1c,d and 2c,d).

During scattering, epithelial markers (for example, E-cadherin and β_4 integrin) are redistributed, but not lost, and mesenchymal markers such as vimentin are not induced (FIG. 2c). Scattering is induced by FGF, SF/HGF or TGF-β alone but is fully reversible after removal of the inducing factors (FIG. 1c and 2c). As TGF-β induces apoptosis in EpH4 cells (in contrast to other cell types used[24–27]), TGF-β-induced scattering

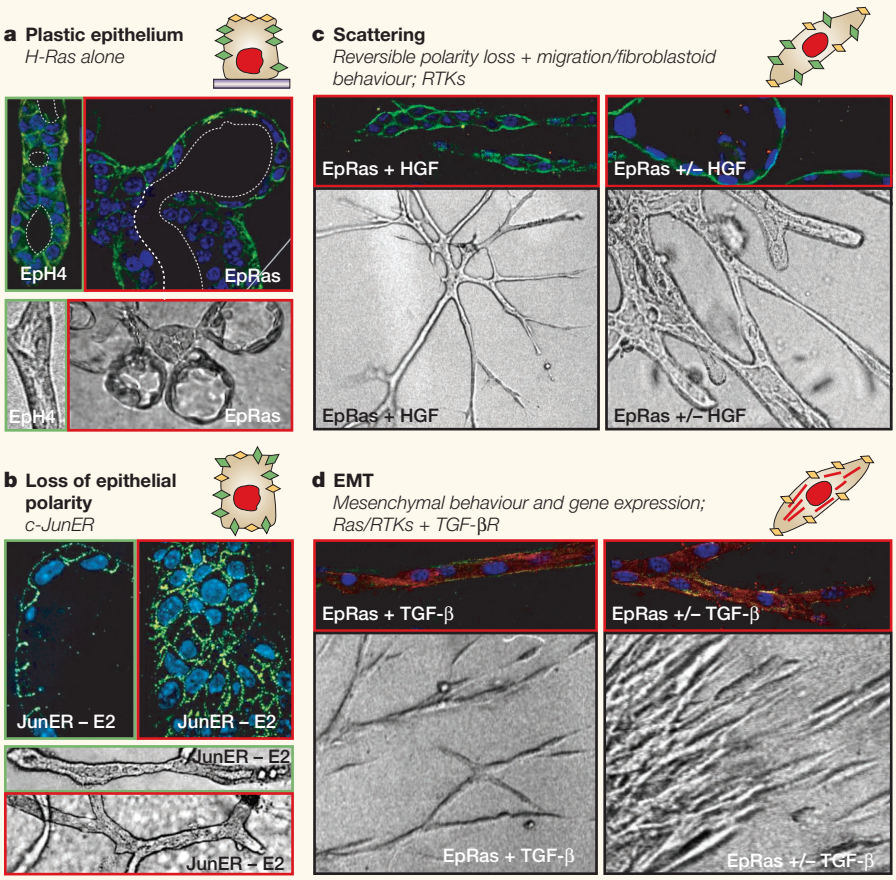


Figure 2 | **Multiparameter analysis of epithelial-plasticity phenotypes in 3D collagen gel cultures.** Four different epithelial-plasticity phenotypes (see text) induced by various external signals and/or oncoproteins in three-dimensional (3D) collagen gels are depicted in bright field and after immunofluorescence analysis (see BOX 2b). The four phenotypes are marked by cell schemes for: (**a**) plastic epithelium (green staining, E-cadherin), (**b**) loss of polarity (green staining, β-catenin), (**c**) scattering (green staining, β_4 integrin; red staining, vimentin) and (**d**) epithelial–mesenchymal transition (EMT; green staining, β_4 integrin), as distinguished by epithelial and mesenchymal markers. Blue staining, nuclear marker. Cell types used and treatments applied to show the respective epithelial-plasticity changes are shown in all panels. In the Jun–oestrogen-receptor fusion (ER) example (**c**), ultrathin sections of the respective structures after staining for the basolateral marker β-catenin are shown instead of confocal micrographs of whole structures. E2, oestradiol; HGF, hepatocyte growth factor; R, receptor; RTKs, receptor tyrosine kinases; TGF-β, transforming growth factor-β. Part **b** is reproduced with permission from REF. 19 © (1996) The Rockefeller University Press. Parts **c** and **d** are reproduced with permission from REF. 18 © (2002) The Rockefeller University Press.

requires protection from apoptosis by potent survival factors such as Bcl-2 or activation of the PI3K pathway[18] (FIG. 1c).

In contrast to scattering, EMT involves loss of E-cadherin, β_4 integrin and ZO-1, and *de novo* expression of mesenchymal markers such as vimentin[6,20,28]. Typically, EMT-inducing signal combinations involve oncogenic Ras and TGF-β, which must be present for prolonged time periods (>4–6 days)[20,22,23,29] (FIG. 1d). Furthermore, EMT persists after withdrawal of these signals, and is stabilized by autocrine TGF-β secretion, so EMT is maintained as a metastable phenotype independent of exogenous factors[17,18,20]. EMT can, however, be reversed by interference with Ras or TGF-β signalling *in vitro*[17,18,20] or *in vivo*[16].

TGF-β cooperates with other signals

One central feature of EMT is the cooperation of TGF-β-family members with receptor tyrosine kinase (RTK)-driven signalling pathways. This cooperation overcomes the tumour-suppressive effects of TGF-β-receptor signalling (cell-cycle arrest and apoptosis induction), so that TGF-β can influence epithelial plasticity and migration/motility, which are crucial for tumour progression and metastasis[30,31]. How does this cooperation function at the molecular level?

Diverse mechanisms contribute to signal cooperation. Cooperation between TGF-β receptors and RTKs or other receptors involves many mechanisms (see REFS 30–32 and references therein), which can be (arbitrarily) divided into four groups (BOX 3). First (group a), TGF-β receptor and RTK signalling converge at the level of transcriptional regulation. This might occur through separate activation of different transcription factors that have similar or opposing actions, or through cooperative mechanisms — for example, complexes of Smads (transcription factors that respond to TGF-β) and other transcription factors. Either mechanism can lead to synergistic or antagonistic target-gene regulation that is dependent on the cellular context. Second (group b), Smad-independent TGF-β-receptor signalling pathways are being discovered that activate or inhibit many signal transducers that are also co-regulated by RTK signalling. Third (group c), several RTKs and other receptors negatively regulate Smad signalling by transcriptional upregulation of inhibitory Smads (Smad6/7). Finally (group d), RTK signalling might induce autocrine production of both TGF-β and other RTK ligands[20,33,34]. TGF-β autoinduces itself[20] and might also contribute to autocrine secretion of platelet-derived growth factor (PDGF) (M. J. *et al.*, unpublished observations). Significantly, this integration between receptor and RTK signalling occurs at many levels, which either enhances the activity of both pathways or leads to intermediate responses owing to the integration of opposing signals (see legend to BOX 3).

***Signal cooperation* in vivo.** Interestingly, cooperation of TGF-β or related factors, such as bone morphogenetic proteins (BMPs), with RTK ligands occurs in most, if not all, normal and pathological situations that involve EMT. Mesoderm formation during gastrulation involves FGFs and BMPs, as well as proteins that positively or negatively regulate the processing, secretion or activity of both factor types[35,36]. TGF-β-family members and many RTK ligands have been reported to regulate trophoblast differentiation[37], neural-crest-cell development[10] and palate development[28]. Significantly, TGF-β and EGF or PDGF also cooperate in matrix accumulation and myofibroblast generation in chronic lung and kidney diseases, and this is aided by inflammatory cytokines such as tumour necrosis factor-α (TNF-α) and various interleukins[11,12]. Finally, tumour progression and metastasis clearly require the cooperation of TGF-β with RTK ligands or downstream oncoproteins *in vitro*[18,20,22] and *in vivo*[29,38,39].

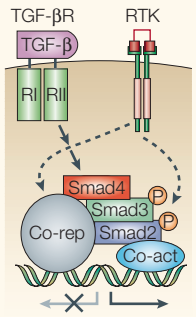
Box 3 | **Cooperation of TGF-β receptors with RTKs: diverse mechanisms**

Schemes **a**–**d** depict regulatory effects of receptor tyrosine kinases (RTKs) and transforming growth factor-β (TGF-β) receptors that are selected for their importance in crosstalk of signals from these two receptor types. Signals from RTKs (dotted arrows/lines) and TGF-β receptors (solid arrows/lines) might either enhance or (more importantly) attenuate each other in a cell-type-specific and signal-specific fashion. Signal integration (mixed solid/dotted arrows/lines) between several of these pathways could provide the molecular basis for the several epithelial-plasticity phenotypes described. At least four groups of mechanisms can be distinguished:

Cooperation through transcriptional regulation (group a)
Smad-dependent transcriptional regulation of TGF-β-target genes is modulated at the transcriptional and post-transcriptional level by RTK signalling — for example, by phosphorylation of Smads, transcription factors or co-regulators — which can also alter the composition/activity of such transcriptional complexes.

Smad-independent signalling (group b)
An increasing number of signalling pathways that do not directly involve Smads might mediate crosstalk to signal transducers that are also regulated by RTKs; for example, extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K), p38 MAPK, ribosomal S6 protein kinase (p70 S6K). In a few cases, new effectors that directly complex to TGF-β receptors (PP2A-Bα, XIAP, DAxx) have been identified, although most of these effectors are still unknown (question marks).

a Transcriptional modulation



TGF-βR
↑p16, p21
↑pro-apoptotic proteins
↑Net1/RhoA/PI3K
↓Cdc25, c-Myc

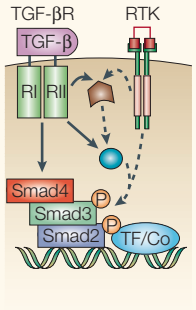
RTKs
↑Ras/ERK/MAPK/AP-1
↑PI3K/anti-apoptotic protein

Stress signals
↑JNK/c-Jun; p38/ATF2

TGF-βR + RTKs
↑E-cad-repression (Snail/ΔEF1)
↑EMT genes

Wnt/E-cadherin signalling
↑β-catenin/LEF/TCF
↓E-cadherin

b Smad-independent signalling



Non-Smad signals

PP2A-Bα/PP2Acat/
p70 S6K-inhibition

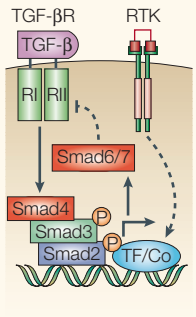
?; RhoA/ROCK/PI3K

XIAP/TAB1/TAK1/
JNK/p38

DAxx/?/JNK/p38
?; Rho/JNK

?, PI3K (p85)

c TGF-βR signal inhibition (I-Smads)



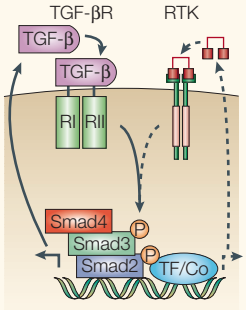
TGFα
NF-κB
pathway

IFNγ
Jak1/Stat1

PDGFR
?/Stat1

EGFR
Unknown

d Autocrine loop induction



RTKs/Ras signalling
TGF-β
EGFR ligands
EGFR/PI3K

TGF-βR signalling
TGF-β
PDGFa/
PDGFR/
Stat1/2
PI3K

TGF-β receptor signal inhibition by induction of inhibitory (I-) Smads (group c)
A surprisingly large number of signalling pathways cause the upregulation of I-Smads (Smad6/7), through mechanisms that are only partially known.

Autocrine loop induction (group d)
RTKs and TGF-β receptors might also cooperate through auto- and/or trans-induction of autocrine loops, involving both RTK ligands and TGF-β family members, which then activate other signal-transduction pathways. Sometimes, these autocrine loops might even operate in cascades (not shown).

Blue circle and red pentagon in part **b** represent signal transduction intermediates. AP-1, activator protein 1; ATF2, activating transcription factor 2; Co, co-repressor or co-activator; Co-act, co-activator; Co-rep, co-repressor; DAxx, Fas-death-domain-associated protein; EGFR, epidermal growth-factor receptor; EMT, epithelial–mesenchymal transition; IFN-γ, interferon-γ; JAK, Janus kinase; JNK, c-Jun amino-terminal kinase; LEF1, lymphoid-enhancer-binding-factor-1; PDGF, platelet-derived growth factor; PP2A, protein phosphatase 2A; PP2A-Bα, the Bα regulatory subunit of PP2A; PP2Acat, catalytic subunit of PP2A; R, receptor; ROCK, Rho kinase; STAT, signal transducer and activator of transcription; NF-κB, nuclear factor κB; TAB1, TAK1-binding protein; TAK1, TGF-β-activated kinase; TCF, T-cell factor; TF, transcription factor; TNF-α, tumour necrosis factor-α; XIAP, X-chromosome-linked inhibitor of apoptosis.

Ras signals in EMT and scattering

Ras functions through numerous effectors, such as Raf1 kinase and PI3K. Raf1 signals through MAPK and ERK kinase (MEK) to ERK/MAPK; and PI3K functions through the Akt/protein kinase B (PKB) pathway[40] (FIG. 3a). The impact of these signalling pathways on epithelial plasticity was analysed in the EpH4 model, using Ras mutants that specifically activate only the Raf1–MEK–ERK/MAPK pathway (V12S35Ras) or the PI3K–Akt/PKB pathway (V12C40Ras). Furthermore, specific inhibitors that block Ras, MEK1 or PI3K (FIG. 3a) were used to interfere with EMT induction or maintenance in EpRas cells. Both approaches showed that EMT requires a hyperactive ERK/MAPK pathway in combination with TGF-β signalling. EpH4 cells transformed with V12S35Ras underwent EMT and expressed vimentin in collagen gels after TGF-β treatment, whereas Ras or MEK1 inhibitors prevented or reverted EMT[18,21] (FIG. 1d). By contrast, protection of EpH4 cells from TGF-β-induced apoptosis by either V12C40Ras-induced PI3K signalling, or Bcl-2 overexpression enabled TGF-β to cause reversible scattering[18] (FIG. 1c and 2c). Significantly, a hyperactive ERK/MAPK pathway (together with basal PI3K signalling) was crucial for EMT, as moderately elevated ERK/MAPK-pathway activity induced few or no effects[18,21].

Are the same pathways also required to induce EMT in other systems? Ras-transformed hepatocytes, Madin–Darby canine

kidney (MDCK) cells that express an oestrogen-inducible Raf1 fusion protein (RafER), and EpH4 cells expressing a similarly inducible c-Fos protein (FosER) all depend on autocrine TGF-β secretion to maintain EMT[22,23,41]. Whereas RafER clearly acted through hyperactive ERK/MAPK signalling, Ras hepatocytes seemed to use the PI3K pathway during EMT induction, but acquired a hyperactive ERK/MAPK pathway during subsequent formation of liver tumours[23] (W. Mikulits, unpublished observations). By contrast, the signalling pathways used by FosER to induce EMT remain to be investigated.

Many other signalling pathways might contribute to the regulation of EMT. For example, β-catenin–lymphoid-enhancer-binding-factor-1 (Lef1) signalling[41,42] cooperates with Smads in transcriptional complexes[43]. EMT might also require the activity of both N-cadherin and α_5 integrin (D. von Bredow *et al.*, unpublished observations). It is not surprising that the regulation of EMT is so complex, as EMT occurs only under rare circumstances *in vivo*[6,8,36] and has to be strictly controlled to prevent abnormal development and tumour progression[16,17].

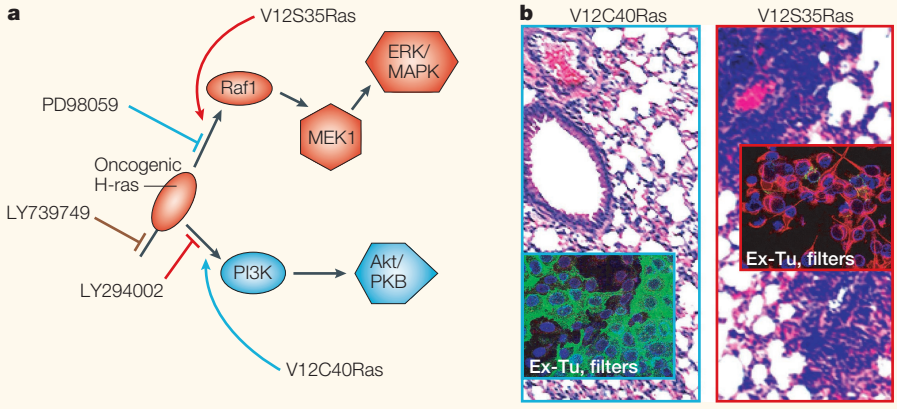


Figure 3 | **Different Ras downstream signalling pathways contribute to distinct tumour phenotypes *in vivo*. a** | Schematic drawing of the Ras downstream signalling pathways that cause either scattering/fast tumour growth (phosphatidylinositol 3-kinase (PI3K) pathway, blue) or epithelial–mesenchymal transition (EMT)/metastasis (extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) pathway, red), in cooperation with transforming growth factor-β. Inhibition of all pathways (by LY739749) or selective inhibition of Ras-downstream pathways (by PD98059 or LY294002 for ERK/MAPK and PI3K, respectively) by appropriate low-molecular-weight inhibitors, as well as selective activation of these pathways by effector-specific Ras mutants (V12S35 and V12C40 for ERK/MAPK and PI3K, respectively) is also depicted. **b** | ERK/MAPK is required for EMT *in vivo* and metastasis. V12S35Ras cells (right panel) but not V12C40Ras cells (left panel) induce lung metastasis after injection into the tail vein of nude mice. Both cell types form tumours in mice (not shown), but cells recultivated on filters from the primary tumours undergo EMT (using the expression of vimentin (red), and the loss of expression of E-cadherin (green) as markers) only in the V12S35 cells and not in the V12C40 cells (see insets). Blue staining, nuclear marker. PKB, protein kinase B; Ex-Tu, ex-tumour; MEK, MAPK and ERK kinase. Part **b** is reproduced with permission from REF. 18 © (2002) The Rockefeller University Press.

Expression profiling

Significant insights into the genes and pathways that are important for the epithelial-plasticity phenotypes described above have been obtained by profiling the expression patterns of polysome-bound messenger RNA[44]. Two groups of EpH4-based cell pairs were analysed: one cell line from each pair was induced to undergo EMT (in the case of three cell pairs) or scattering (in the case of two cell pairs) using various different oncogenes/factors. Expression profiles of the induced and non-induced cell lines from each pair were compared to identify either EMT- or scattering-specific genes[54] (BOX 2c). Two cell pairs analysed before or after transformation with Ras or Bcl2 served as controls. The expression profiles obtained for the three EMT cell pairs were similar, but strikingly different to those obtained by the two scattering cell pairs. Ninety-two genes were specifically upregulated in EMT, whereas more than 300 upregulated genes were specific for scattering, as well as being upregulated in plastic epithelium. Interestingly, many genes that were strongly associated with EMT are known to promote tumorigenesis, including both key regulators and components of entire signalling pathways that are important for cell proliferation, epithelial polarity and motility/invasiveness. For instance, six genes involved in PDGF-receptor signalling were upregulated during EMT. Functional analysis confirmed that TGF-β induces an autocrine PDGF loop during EMT. This autocrine PDGF signalling is essential for EMT: it functions through PDGF-receptor-mediated hyperactivation of the PI3K pathway beyond the level that is induced by oncogenic Ras, a process that is required for complete protection of EpRas cells from TGF-β-induced apoptosis[54]. This established that scattering and EMT are separate, molecularly distinct processes in epithelial plasticity. It remains to be established whether any of these 'EMT genes' also discriminate between benign and metastatic human tumours.

Epithelial plasticity: *in vivo* relevance?

Is EMT an* in vitro *correlate of metastasis? To address the relevance of epithelial plasticity for metastatic potential, EpH4 cells expressing wild-type or Ras-effector mutant proteins (V12S35Ras and V12C40Ras) were injected into mice. All tumorigenic cell types that could undergo EMT (EpRas, V12S35Ras and Ras hepatocytes) showed metastatic ability[18,23]. By contrast, the tumours induced by V12C40Ras cells failed to metastasize, in line with their inability to undergo TGF-β-induced EMT *in vitro*[18,21] (FIG. 3b). Therefore, EMT represents a faithful *in vitro* correlate of metastasis, consistent with its role in development during which cells digest and traverse through basement membranes and migrate to distant sites in the embryo[6]. It is unclear, however, whether or not human tumour cells that only undergo scattering, or show unrelated epithelial-plasticity phenotypes[45], can metastasize without (at least transiently) undergoing EMT.

EMT and tumour growth rate: independent regulation? Activation/overexpression of ErbB2 (also known as HER2/neu)[38,46] or Ras signalling through polyoma middle T (REF. 47) cause mammary-gland hyperplasias or tumours, indicating that these signals cause tumour-cell hyperproliferation. This *in vivo* effect was recently reproduced in culture using 3D collagen gels. In this system, Ras or ErbB2-transformed EpH4 cells proliferated much faster than EpH4 control cells[21]. Both hyperproliferation in 3D cultures and rapid tumour growth were dependent on enhanced PI3K signalling, as shown using EpH4 cells that expressed V12C40Ras. By contrast, tumours that arose from moderate activation of the ERK/MAPK pathway in V12S35Ras-EpH4 cells showed delayed onset and/or reduced growth rate, but still underwent EMT *in vivo*. Consequently, EMT and tumour-cell hyperproliferation

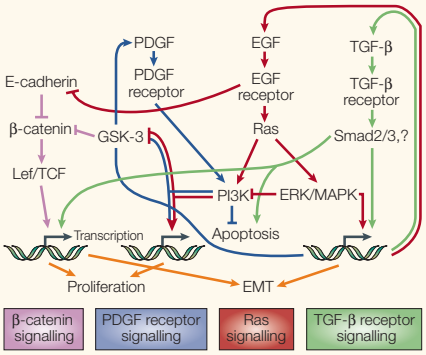


Figure 4 | **Signalling networks regulating carcinoma progression.** This highly simplified schematic depicts how distinct signalling pathways downstream of receptor tyrosine kinases, receptor serine/threonine kinases and adhesion molecules might regulate tumour-cell proliferation, apoptosis protection and epithelial–mesenchymal (EMT)/metastasis through multiple positive and negative interactions between receptors, signal transducers and/or transcriptional regulators. Furthermore, important feedback regulation is provided by several autocrine loops that involve both tyrosine and serine/threonine kinase ligands/receptors. Components of and input by the different signalling pathways are marked by distinct colours, as shown at the bottom of the figure. The orange colour represents phenotypical changes that result from the various signal interplays. EGF, epidermal growth factor; ERK, extracellular signal-regulated kinase; GSK-3, glycogen synthase kinase 3; PDGF, platelet-derived growth factor; MAPK, mitogen-activated protein kinase; PI3K, phosphatidylinositol 3-kinase; TCF, T-cell factor; TGF-β, transforming growth factor-β.

might represent independent processes that are regulated by different signal-transduction pathways downstream of Ras[21].

***ERK/MAPK plus TGF-β: relevance for metastasis* in vivo?** Several *in vivo* animal models confirm that metastasis requires a hyperactive ERK/MAPK pathway as well as TGF-β-receptor signalling. Transgenic TGF-β expression attenuated the formation of Ras-induced, benign skin tumours (papillomas) in mice, but strongly stimulated their progression to spindle-cell carcinomas[29], which metastasize in a TGF-β-dependent manner[48]. A similar cooperation of TGF-β with ErbB2 (REF. 38) to enhance *in vivo* EMT and metastasis was recently observed in a transgenic mammary-carcinoma mouse model (S. G., unpublished observations). Furthermore, overexpression of dominant-active Smad2 caused cell lines derived from Ras-transformed squamous carcinomas to undergo EMT and form metastatic tumours[49]. Nevertheless, probably owing to the aforementioned difficulties in clearly detecting EMT, explicit evidence for the requirement of Ras plus TGF-β-induced EMT in primary human tumours is still missing.

TGF-β: tumour suppression or promotion? Several findings in mice and humans clearly show that TGF-β signalling is required during late tumour progression/metastasis, despite its tumour-suppressive role in early tumorigenesis. For example, certain rare familial colon tumours often lose TGF-β receptor II, but have a much better prognosis than sporadic colon tumours that retain TGF-β signalling[50]. Furthermore, mice that lack the adenomatosis polyposis coli (Apc) protein develop highly metastatic colon tumours, but this metastatic potential is lost in *Apc*$^{-/-}$ mice that also lack Smad4 (REF. 51). Furthermore, cell-autonomous metastatic potential of the murine colon carcinoma cell line CT26 was completely suppressed by a dominant-negative TGF-β receptor II (REF. 17), and similar findings were obtained with metastatic breast tumour cells[52]. Finally, inhibition of TGF-β signalling by a soluble TGF-β-receptor-II–Fc fusion protein reduced lung metastasis in mouse mammary tumour virus (MMTV)-ErbB2 mice injected with metastatic cell lines[39,53].

Implications and future directions

EMT and metastasis are complex developmental processes that involve significant reprogramming of gene expression. This is regulated by many external signals that eventually — through a complex signalling network — cause cell-fate changes and key alterations in cell behaviour. Many signalling pathways — ERK/MAPK, PI3K, Smads, β-catenin, and at least two autocrine growth-factor loops (TGF-β, PDGF) — contribute to this network (FIG. 4).

Perturbing the regulation of this complex signalling network during tumour progression probably requires selection of tumour cells for multiple, sequential alterations. In one possible scenario, epithelial cells could gain partial resistance to TGF-β-mediated cell-cycle arrest and apoptosis by hyperactivation of PI3K signalling through chronically activated EGF receptor 1 or 2. In the surviving cells, loss of the cell-cycle-dependent kinase (CDK) inhibitor INK4b (also known as p15) might abolish the TGF-β-induced cell-cycle arrest. Additionally, mutation or amplification of ErbB1, ErbB2 or Ras will stabilize and further increase TGF-β resistance, whereas the hyperactive ERK/MAPK signalling that is induced by these mutations, together with TGF-β — which is initially produced by the tumour stroma[20] or other sources — will induce EMT. EMT will lead to autocrine secretion of both TGF-β and PDGF, probably owing to activation of the mesenchymal gene-expression programme. Whereas TGF-β stabilizes EMT, PDGF might increase TGF-β resistance to apoptosis to a level that is not possible through Ras alone. Finally, autocrine signalling through EGF, TGF-β or PDGF could enhance β-catenin signalling, which might contribute to EMT. This corresponds to the essential function of β-catenin in mesoderm formation (FIG. 4) (see above for references).

It is still unclear whether or not scattering and EMT are distinct, clearly definable cellular states or just represent more frequently observed states within a continuum of epithelial plasticity. This question is being addressed at present by further expression-profiling studies (J. Gotzmann and W. Mikulits, unpublished observations; A. Eger, J. Wolf and R. Foisner, unpublished observations). Preliminary results from such screens agree with the idea that scattering could be an acute, reversible process that is caused by short-term signals, whereas long-term action of the same signals might cause EMT. In pathological situations, short-term stimulation might occur during wound healing, whereas long-term stimuli could be involved in chronic inflammation.

A final, important question that is particularly relevant to therapeutic approaches is whether TGF-β-induced EMT occurs in most or even all metastatic carcinomas. Several genetic mouse tumour models in which TGF-β signalling is enhanced or interfered with, support EMT as a general mechanism in metastasis. It remains to be clarified, however, whether the residual metastases that continue to arise after TGF-β-receptor signalling has been inhibited are caused by incomplete inhibition of that pathway, or whether mechanisms that are independent of TGF-β-receptor signalling occur.

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1. Hunter, T. Oncoprotein networks. *Cell* **88**, 333–346 (1997).
2. Hanahan, D. & Weinberg, R. A. The hallmarks of cancer. *Cell* **100**, 57–70 (2000).
3. Elenbaas, B. & Weinberg, R. A. Heterotypic signaling between epithelial tumor cells and fibroblasts in carcinoma formation. *Exp. Cell Res.* **264**, 169–184 (2001).

4. Thiery, J. P. Epithelial–mesenchymal transitions in tumour progression. *Nature Rev. Cancer* **2**, 442–454 (2002).
5. Cordon-Cardo, C. & Prives, C. At the crossroads of inflammation and tumorigenesis. *J. Exp. Med.* **190**, 1367–1370 (1999).
6. Hay, E. D. An overview of epithelio–mesenchymal transition. *Acta Anat.* **154**, 8–20 (1995).
7. Viebahn, C. Epithelio–mesenchymal transformation during formation of the mesoderm in the mammalian embryo. *Acta Anat.* **154**, 79–97 (1995).
8. Sun, D., Baur, S. & Hay, E. D. Epithelial–mesenchymal transformation is the mechanism for fusion of the craniofacial primordia involved in morphogenesis of the chicken lip. *Dev. Biol.* **228**, 337–349 (2000).
9. Martinez-Alvarez, C. *et al.* Medial edge epithelial cell fate during palatal fusion. *Dev. Biol.* **220**, 343–357 (2000).
10. Duband, J. L., Monier, F., Delannet, M. & Newgreen, D. Epithelium–mesenchyme transition during neural crest development. *Acta Anat.* **154**, 63–78 (1995).
11. Knight, D. Epithelium–fibroblast interactions in response to airway inflammation. *Immunol. Cell Biol.* **79**, 160–164 (2001).
12. Alpers, C. E. *et al.* Localization of SPARC in developing, mature, and chronically injured human allograft kidneys. *Kidney Int.* **62**, 2073–2086 (2002).
13. Yang, J. & Liu, Y. Blockage of tubular epithelial to myofibroblast transition by hepatocyte growth factor prevents renal interstitial fibrosis. *J. Am. Soc. Nephrol.* **13**, 96–107 (2002).
14. Petersen, O. W. *et al.* Epithelial to mesenchymal transition in human breast cancer can provide a nonmalignant stroma. *Am. J. Pathol.* **162**, 391–402 (2003).
15. Putz, E. *et al.* Phenotypic characteristics of cell lines derived from disseminated cancer cells in bone marrow of patients with solid epithelial tumors: establishment of working models for human micrometastases. *Cancer Res.* **59**, 241–248 (1999).
16. Brabletz, T. *et al.* Variable β-catenin expression in colorectal cancers indicates tumor progression driven by the tumor environment. *Proc. Natl Acad. Sci. USA* **98**, 10356–10361 (2001).
17. Oft, M., Heider, K. H. & Beug, H. TGFβ signalling is essential for carcinoma cell invasiveness and metastasis. *Curr. Biol.* **8**, 1243–1252 (1998).
18. Janda, E. *et al.* Ras and TGFβ cooperatively regulate epithelial cell plasticity and metastasis: dissection of Ras signaling pathways. *J. Cell Biol.* **156**, 299–313 (2002).
19. Fialka, I. *et al.* The estrogen-dependent c-JunER protein causes a reversible loss of mammary epithelial cell polarity involving a destabilization of adherens junctions. *J. Cell Biol.* **132**, 1115–1132 (1996).
20. Oft, M. *et al.* TGFβ1 and Ha-Ras collaborate in modulating the phenotypic plasticity and invasiveness of epithelial tumor cells. *Genes Dev.* **10**, 2462–2477 (1996).
21. Janda, E., Litos, G., Grunert, S., Downward, J. & Beug, H. Oncogenic Ras/Her-2 mediate hyperproliferation of polarized epithelial cells in 3D cultures and rapid tumor growth via the PI3K pathway. *Oncogene* **21**, 5148–5159 (2002).
22. Lehmann, K. *et al.* Raf induces TGFβ production while blocking its apoptotic but not invasive responses: a mechanism leading to increased malignancy in epithelial cells. *Genes Dev.* **14**, 2610–2622 (2000).
23. Gotzmann, J. *et al.* Hepatocytes convert to a fibroblastoid phenotype through the cooperation of TGF-β1 and Ha-Ras: steps towards invasiveness. *J. Cell Sci.* **115**, 1189–1202 (2002).
24. Miettinen, P. J., Ebner, R., Lopez, A. R. & Derynck, R. TGF-β induced transdifferentiation of mammary epithelial cells to mesenchymal cells: involvement of type I receptors. *J. Cell Biol.* **127**, 2021–2036 (1994).
25. Piek, E., Moustakas, A., Kurisaki, A., Heldin, C. H. & ten Dijke, P. TGF-β type I receptor/ALK-5 and Smad proteins mediate epithelial to mesenchymal transdifferentiation in NMuMG breast epithelial cells. *J. Cell Sci.* **112**, 4557–4568 (1999).
26. Bakin, A. V., Tomlinson, A. K., Bhowmick, N. A., Moses, H. A. & Arteaga, C. L. Phosphatidylinositol 3-kinase function is required for transforming growth factor β-mediated epithelial to mesenchymal transition and cell migration. *J. Biol. Chem.* **275**, 36803–36810 (2000).
27. Bhowmick, N. A. *et al.* Transforming growth factor-β1 mediates epithelial to mesenchymal transdifferentiation through a RhoA-dependent mechanism. *Mol. Biol. Cell* **12**, 27–36 (2001).
28. Sun, D., Vanderburg, C. R., Odierna, G. S. & Hay, E. D. TGFβ3 promotes transformation of chicken palate medial edge epithelium to mesenchyme *in vitro*. *Development* **125**, 95–105 (1998).
29. Cui, W. *et al.* TGFβ1 inhibits the formation of benign skin tumors, but enhances progression to invasive spindle carcinomas in transgenic mice. *Cell* **86**, 531–542 (1996).
30. Derynck, R., Akhurst, R. J. & Balmain, A. TGF-β signaling in tumor suppression and cancer progression. *Nature Genet.* **29**, 117–129 (2001).
31. Ten Dijke, P., Goumans, M. J., Itoh, F. & Itoh, S. Regulation of cell proliferation by Smad proteins. *J. Cell. Physiol.* **191**, 1–16 (2002).
32. Attisano, L. & Wrana, J. L. Signal transduction by the TGF-β superfamily. *Science* **296**, 1646–1647 (2002).
33. Heldin, C. H. & Westermark, B. Mechanism of action and *in vivo* role of platelet-derived growth factor. *Physiol. Rev.* **79**, 1283–1316 (1999).
34. Schulze, A., Lehmann, K., Jefferies, H. B., McMahon, M. & Downward, J. Analysis of the transcriptional program induced by Raf in epithelial cells. *Genes Dev.* **15**, 981–994 (2001).
35. Beddington, R. S. & Robertson, E. J. Axis development and early asymmetry in mammals. *Cell* **96**, 195–209 (1999).
36. Ciruna, B. & Rossant, J. FGF signaling regulates mesoderm cell fate specification and morphogenetic movement at the primitive streak. *Dev. Cell* **1**, 37–49 (2001).
37. Vicovac, L. & Aplin, J. D. Epithelial–mesenchymal transition during trophoblast differentiation. *Acta Anat.* **156**, 202–216 (1996).
38. Muller, W. J., Sinn, E., Pattengale, P. K., Wallace, R. & Leder, P. Single-step induction of mammary adenocarcinoma in transgenic mice bearing the activated *c-neu* oncogene. *Cell* **54**, 105–115 (1988).
39. Muraoka, R. S. *et al.* Blockade of TGF-β inhibits mammary tumor cell viability, migration, and metastases. *J. Clin. Invest.* **109**, 1551–1559 (2002).
40. Downward, J. Mechanisms and consequences of activation of protein kinase B/Akt. *Curr. Opin. Cell Biol.* **10**, 262–267 (1998).
41. Eger, A., Stockinger, A., Schaffhauser, B., Beug, H. & Foisner, R. Epithelial mesenchymal transition by c-Fos estrogen receptor activation involves nuclear translocation of β-catenin and upregulation of β-catenin/lymphoid enhancer binding factor-1 transcriptional activity. *J. Cell Biol.* **148**, 173–188 (2000).
42. Stockinger, A., Eger, A., Wolf, J., Beug, H. & Foisner, R. E-cadherin regulates cell growth by modulating proliferation-dependent β-catenin transcriptional activity. *J. Cell Biol.* **154**, 1185–1196 (2001).
43. Labbe, E., Letamendia, A. & Attisano, L. Association of Smads with lymphoid enhancer binding factor 1/T cell-specific factor mediates cooperative signaling by the transforming growth factor-β and wnt pathways. *Proc. Natl Acad. Sci. USA* **97**, 8358–8363 (2000).
44. Pradet-Balade, B., Boulme, F., Beug, H., Mullner, E. W. & Garcia-Sanz, J. A. Translation control: bridging the gap between genomics and proteomics? *Trends Biochem. Sci.* **26**, 225–229 (2001).
45. Vecsey-Semjen, B. *et al.* Novel colon cancer cell lines leading to better understanding of the diversity of respective primary cancers. *Oncogene* **21**, 4646–4662 (2002).
46. Andrechek, E. R. *et al.* Amplification of the *neu/erbB-2* oncogene in a mouse model of mammary tumorigenesis. *Proc. Natl Acad. Sci. USA* **97**, 3444–3449 (2000).
47. Webster, M. A. *et al.* Requirement for both Shc and phosphatidylinositol 3′ kinase signaling pathways in polyomavirus middle T-mediated mammary tumorigenesis. *Mol. Cell Biol.* **18**, 2344–2359 (1998).
48. Weeks, B. H., He, W., Olson, K. L. & Wang, X. J. Inducible expression of transforming growth factor β1 in papillomas causes rapid metastasis. *Cancer Res.* **61**, 7435–7443 (2001).
49. Oft, M., Akhurst, R. J. & Balmain, A. Metastasis is driven by sequential elevation of H-ras and Smad2 levels. *Nature Cell Biol.* **4**, 487–494 (2002).
50. Jacob, S. & Praz, F. DNA mismatch repair defects: role in colorectal carcinogenesis. *Biochimie* **84**, 27–47 (2002).
51. Takaku, K. *et al.* Intestinal tumorigenesis in compound mutant mice of both *Dpc4* (*Smad4*) and *Apc* genes. *Cell* **92**, 645–656 (1998).
52. McEarchern, J. A. *et al.* Invasion and metastasis of a mammary tumor involves TGF-β signaling. *Int. J. Cancer* **91**, 76–82 (2001).
53. Yang, Y. A. *et al.* Lifetime exposure to a soluble TGF-β antagonist protects mice against metastasis without adverse side effects. *J. Clin. Invest.* **109**, 1607–1615 (2002).
54. Jechlinger, M. *et al*. Expression profiling of epithelial plasticity in tumour progression. *Oncogene* (in the press).

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α_5 integrin | β_4 integrin | BMPs | FGF | H-Ras | MAPK | MEK | PDGF | PI3K | Rac | Ras | Rho | Smads | TGF-β

Swiss-Prot: http://www.expasy.ch/
Bcl2 | E-cadherin | EGF | ErbB2 | HGF | Lef1 | N-cadherin | PKB | Raf1 | Smad2 | Smad4 | Smad6 | Smad7 | TNF-α | transforming growth factor α | vimentin | ZO-1

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