Epithelial-mesenchymal transition: a cancer researcher's conceptual friend and foe.

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Abbreviation: EMT: Epithelial-mesenchymal transition

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Abstract

Epithelial-mesenchymal transition (EMT) describes a series of rapid changes in cellular phenotype. Epithelial cells loosen cell-cell adhesion structures, change their polarity, modulate their cytoskeletal organization and typically become isolated, motile, and resistant to anoikis. EMT is often applied to distinct biological events as if it were a single conserved process, but in fact these processes can vary in intensity from transient depolarization to total cellular reprogramming as found by transcriptional analysis. Adjustments are therefore necessary when applying the term EMT to tumor progression. Based on clinical observations, it is more appropriate in most cases to describe the emergence of an "EMT-like" phenotype during tumor progression. This does not imply complete trans-differentiation but emphasizes the intermediary phenotype associated with tumor cell renewal and adaptation to specific microenvironments. Here, we categorize the various EMT-like phenotypes found in human carcinomas that, depending on the tumor type, may or not represent analogous stages in tumor progression. We based these categories on global tumor phenotype. Tumor microenvironment associated with stromal reactions, hypoxia, paucity of nutrients, impaired differentiation and that activation of various EMT-associated pathways, modulate overall tumor phenotype and lead to tumor heterogeneity.

The epithelial-mesenchymal transition concept: Epithelial-mesenchymal transition (EMT) describes a rapid and often reversible change of cell phenotype. EMT was originally defined in the context of cellular remodeling that occurs during heart morphogenesis [1], and has been applied to a range of events, including mesoderm and neural crest formation [2]. The reverse process, mesenchymal-epithelial transition also occurs during development [3]. During EMT, epithelial

cells loosen their characteristic cell-cell adhesion structures, change their polarity, modulate the organization of their cytoskeletal systems, switch expression from keratin- to vimentin-type intermediate filaments, and become isolated, motile, and resistant to anoikis [4 5].

EMT bears at least a superficial resemblance to the steps in the transition of normal to metastatic cells during epithelial tumor progression. That the concept of EMT was relevant to tumor progression was refined and explored *in vitro*, most notably by E.D. Hay, using epithelial cell models; these cells could be transformed into individualized motile cells through manipulations using growth factors or extracellular matrix components [6]. Original EMT models include the transformation of lens epithelial cells growing in collagen gels [7], NBT-II carcinoma cells responding to fibroblast growth factor (FGF) [8] and MDCK cells responding to hepatocyte growth factor/scatter factor (HGF/SF) [9-11]. Over the years, many more models have been described, and a number of genes have been implicated in EMT-like behavior of tumor cell [12]. As is often the case, genes active (and activated) during the course of carcinoma progression and metastasis are also active in other processes, specifically during early embryogenesis, tissue morphogenesis, wound healing [2, 13-15]. This has lead to the idea that EMT, as it occurs in the course of developmental events, is “reactivated" during tumor progression. The popularity of this idea is suggested by the fact that a recent search on "epithelial-mesenchymal transition" and "cancer" using PubMed (NCBI, NIH) yielded more than 463 references. On the other hand, the assumption that EMT is actually relevant to cancer progression has raised serious objections from pathologists who points out the lack of direct clinical evidence for EMT [16]. A re-evaluation of the literature suggests that there are good reasons to make a clear distinction between EMT senso stricto and the EMT-like phenotype observed in carcinoma.

Relevance of EMT in the cancer environment: By definition, EMT can apply only to epithelial-derived cancers (carcinomas), which account for 90% of human tumors. To intravasate and metastasize, epithelial cells must at least transiently down-regulate their cell-cell adhesion structures [14]. The loss of cell-cell adhesion, however, does not make an EMT! Perhaps the major obstacle in identifying EMT-specific events is the lack of definitive markers for post-EMT cells. For example keratin expression is routinely used by pathologists to identify epithelial-derived carcinoma cells [17], but cannot be used to identifying post-EMT cells since these cells should (if an actual EMT event were taking place) no longer express keratin. In fact, post-EMT epithelial cells will be phenotypically similar to (normal) vimentin-expressing stromal cells. Several groups have attempted to decipher the clonal origins of epithelial and mesenchymal components of tumors by comparative genotypic analysis using microsatellite markers [22-25]. In fact, some less common tumors such as phyllodes tumors of the prostate were found to combine stromal and epithelial components with distinct clonal origins [18]. However, this strategy has been problematic when applied to more prevalent mammary invasive ductal carcinomas: transformed stroma cells share chromosomal rearrangements with transformed epithelial cells, suggesting common origin, but also contain stroma specific rearrangements [19-21]. A clearer situation is found in carcinosarcoma, rare tumors mostly described in uterus and lung, that display a mix of epithelial and mesenchymal cell types. Most studies indicate that these tumors are derived from a common epithelial progenitor that gives rise to a mesenchymal subpopulation presumably through an EMT-like process.

"EMT-like" phenotypes in cancer: There is an important distinction to make based on the poorly differentiated state of many carcinoma cells. Their phenotype may reflect a dedifferentiation rather than a trans-differentiation process, such as occurs during EMT. From

this perspective, transformed epithelial progenitors/stem cells may simply fail to differentiate normally, rather than differentiate into mesenchymal cells. If this is the case, the use of the term EMT is inappropriate and confusing, since it is misleading about the origin of the cells (Fig. 1).

To address this issue, we suggest using the term "EMT-like" as a more descriptive and accurate term to describe the phenotypes of epithelial cancers, since it does not imply a specific mechanism or the origin of the cells described. We propose three functional criteria to define "EMT-like" phenotypes in human carcinomas: a) state of cell polarization, b) state of cell cohesiveness and c) intermediate filament protein expression. Based on these criteria we define the following four EMT-like phenotypes :

Phenotype 0: Differentiated tumor cells with preserved epithelial structure and cell polarity,

Phenotype 1: Most tumor cells display cellular depolarization [15], but retain cohesive cell-cell contacts and keratin expression.

Phenotype 2: There is loss of cell-cell adhesion in most tumor cells, but the cells still express keratins.

Phenotype 3: There is loss of keratin expression and substantial expression of vimentin in most tumor cells.

Considering the phenotypic heterogeneity of most human tumors, this classification does not preclude that some tumor area may show distinct phenotypic features. That said, most tumors do display a dominant phenotype which is used by pathologist for typing. Applying this scheme to human carcinoma types we have evaluated all major human carcinoma types, based on reference works [26, 27], original articles [28-30], and our personal observations on breast and colon carcinomas typed and identified by the pathology department at CRLC Val d'Aurelle

(Montpellier, France)(Table 1). Some of the criteria we are suggesting here for "EMT-like" phenotyping are used in routine by pathologists. For example, expression of E-cadherin, a major player in epithelial cell-cell adhesion, is used to differentiate breast invasive ductal carcinoma which express E-cadherin protein and invasive lobular carcinoma that do not [31, 32]. In summary, there are limited but therapeutically significant numbers of carcinomas with phenotypes 2 and 3, involving poorly differentiated cells that may remain partially cohesive.

EMT-like behavior and tumor heterogeneity: Typically, most carcinomas display histological heterogeneity. We and others [14]; [33] have suggested that only a small percentage of tumor cells ever undergo a total (corresponding to Phenotype 3) and that it is these cells that are presumably, the source of actively metastatic cells. Perhaps the best-documented example of this is the invasive front in colorectal carcinomas. Cells in this region usually express a less differentiated "progenitor/stem cell" phenotype [34] together with nuclear β-catenin, indicative of active canonical Wnt signaling. They are thought to play a critical role in tumor invasive mechanism.

A link between progenitor/stem cells and EMT-like grade is expected based on the immature epithelial phenotype of precursor cells [34]. Many genes involved in progenitor/stem cell maintenance also appear to be involved in the regulation of cell motility, EMT and EMT-like phenotypes. Among these genes CD24, CD44, CD49/Integrin α6, CD29/Integrin β1, and Slug (Snail2) constitute good examples [35-39]. Recently, work from R. Weinberg's laboratory has emphasized molecular links between EMT process and emergence of "stemness" [40]. This is interesting because stemness is distinct from mesenchymal behavior.

Epithelial cells initiate migration by becoming "activated". Activation is considered a "metastable phenotype" [41] that combines incomplete differentiation, cell motility and some cell-cell cohesiveness. It can be considered "EMT-like". For example, during wound healing,

immature basal keratinocytes are activated by changes in the local microenvironment and express such a metastable phenotype [38]. Their migration involves cohort migration, in which cells migrate as groups, not isolated cells. Similarly, during mammary gland tubulogenesis, cells located at the tip of growing tubule terminal end buds (cap cells) migrate as a group entity and express specific cadherins (P-cadherin) and migrate as a group. Interfering with cell-cell adhesion disrupts rather than enhances the migratory pattern [42]. This type of behavior is similar to that displayed by carcinoma cells expressing a metastable phenotype; it explains the cellular chords and partially differentiated tubules found in mammary invasive ductal carcinoma. Total lack of cohesiveness between carcinoma cells, more representative of EMT, represents a distinct mode of invasion, present, for example, in infiltrating lobular carcinoma. This type of invasion is more insidious: while not inherently more invasive than invasive ductal carcinomas, it tends to be detected later during tumor progression, resulting in a poorer prognosis.

Old and new pathways: what happened to EMT master genes? In vitro and in vivo model systems have allowed the characterization of various pathways leading to EMT and EMT-like phenotypes. Such pathways are referred to as EMT pathways in this review, without assuming functional specificity. Five main pathways have been found to trigger EMT-associated process [13, 14, 43]: Tyrosine kinase receptors (EGF, FGF, HGF, PDGF, IGF), integrins, Wnt, NFκB and TGFβ pathways. These pathways involve Akt, GSK3, Rho-GTPases and SMAD signaling pathways. A distinct EMT pathway has been recently described involving the protein tyrosine phosphatase Pez [44]. In direct association with cancer progression, several molecules including ILEI [12, 45], RKIP [46], and CXCR4 [47] appear to control EMT-like phenotypes and tumor metastasis in mouse models [13, 14]. The on-set of both EMT and EMT-like events are associated with loss of cellular polarity, partial to total destabilization of cell-cell junctions, remodeling and replacement

of cytoskeletal components, the onset of cell motility and the suppression of apoptosis. Transcriptional down regulation of junctional components accompanies the EMT process in several systems [13, 48] and may be the cause or an effect of EMT-like events.

Down-regulation of E-cadherin is linked to cell-cell dissociation and invasion in pancreas, prostate and mammary gland mouse cancer models [49, 50]. Specific transcription factors, in particular Snail (Snail1), Slug (Snail2), Twist, SIP1/Zeb and E47, negatively regulate E-cadherin expression [51, 52], and presumably display overlapping functional redundancy, in part through their common recognition of E-box sequences. These factors appear to be involved in most physiological EMT situations. Their over-expression in epithelial cell lines usually induces an EMT [48, 51-53]. However, their specificity is clearly not restricted to E-cadherin regulation and EMT process. For example, members of the Snail family have been shown to be involved in cell motility, proliferation control, differentiation and apoptotic regulation in vivo and in cell models [54-59]. At the same time, detailed mechanism(s) of their effects remain unclear since cellular co-expression of Snail and E-cadherin has been described in breast and colon carcinomas by several groups [33, 60].

Distinct pathways inducing EMT have been uncovered recently, emphasizing functional links between EMT-like phenotypes and inductive pathways specifically activated during tumor growth and progression (Fig. 2). Tumor cell growth requires an increase in local vasculature to provide metabolites and oxygen. Cells adjust to a nutritionally impoverished and hypoxic environment by activating specific pathways, associated with hypermetabolism [61], glycolysis and resistance to acidosis-induced toxicity, and neoangiogenesis. Hypoxia genes have been found to be expressed locally within solid tumors, probably contributing to tumor heterogeneity [62]. The link between hypoxia and EMT has been recently strengthened by the observed activation of Snail and Twist expression by HIF-1, a key hypoxia effector [63-65]. Another hypoxia-related gene,

lysyl oxydase, was found to interact directly with Snail, but the functional significance of this interaction has been disputed [66, 67].

Another specific feature of tumor microenvironment is the stromal reaction through which epithelial-mesenchymal interactions activate or regulate several pathways involving integrins, cytokines and growth factors that are critical for tumor growth and metastasis [68]. Inflammatory cells play a major role in secreting activating factors, and NF-κB, a key regulator of the inflammatory response has been found to regulate Slug, Snail, and Twist [69], unpublished observation). A putative role of macrophages in dragging individualized cells from mammary tumors into the bloodstream has recently been suggested in striking movies [70].

In conclusion, EMT has often been tacitly assumed to be a single conserved process, but this is by no means unambiguously established. What is clear is that EMT and EMT-like processes are influenced by the origin of the cells under consideration. Based on clinical observations, it appears more appropriate in most cases to describe the emergence of an "EMT-like" phenotype during tumor progression. This descriptive term does not necessarily imply an active dedifferentiation process but emphasizes an intermediary phenotype resulting from tumor cell renewal and adaptation to specific microenvironments. It clearly emphasizes the importance of better understanding cell population kinetics, survival and differentiation mechanisms during tumor growth and metastasis.

Figure legends

Fig 1. EMT, "EMT-like" phenotype and tumor progression in breast carcinoma. EMT-like phenotype can be interpreted in several ways. It can result from the transformation of

normally differentiated epithelial cells followed by an EMT process, generating tumor cells with poorly differentiated features (a). Conversely, "EMT-like" phenotype can result from a lack of differentiation by stem/progenitor cells, resulting in cells expressing a partially differentiated phenotype: P1 (Most tumor cells characterized by cellular depolarization but retaining cohesive cell-cell contacts and keratin expression), P2 (Loss of cell-cell adhesion in most tumor cells, still expressing keratins) or P3 (Loss of keratin and substantial expression of vimentin). These early progenitors express a metastable phenotype but do not reach an epithelial differentiated state (b). In both situations, tumor microenvironment controls cell phenotype through several tumor-specific pathways being investigated.

Fig. 2. Tumor-specific changes converge in inducing EMT phenotype. Most pathways controlling EMT-like behavior in cancer cells can be linked to specific environmental changes occurring during tumor growth and progression. Tumor cells adapt to an impoverished and hypoxic environment by activating alternative pathways to adjust to hypoxia (a) and adopting hypermetabolism (b). Tumor cells typically express a partially differentiated phenotype suggesting impairment in differentiation pathways (c). Finally, another specific feature of tumor microenvironment is the stromal reaction. Stromal and inflammatory cells play a major role in secreting activating factors and controlling tumor cells motility (d).

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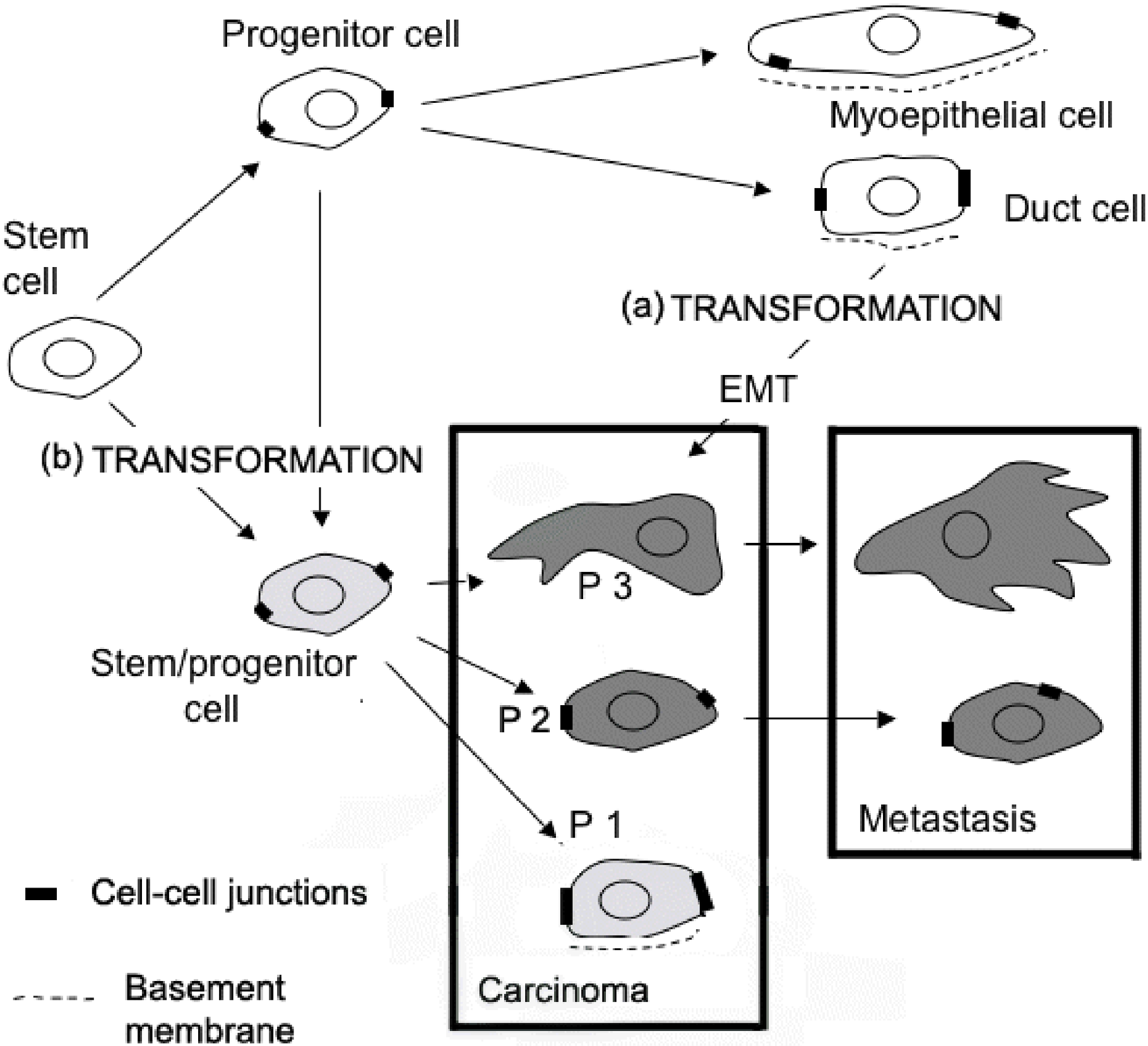
Progenitor cell
Myoepithelial cell
Duct cell
Stem cell
(a) TRANSFORMATION
EMT
(b) TRANSFORMATION
P 3
Stem/progenitor cell
P 2
P 1
Metastasis
Cell-cell junctions
Basement membrane
Carcinoma

Fig. 1

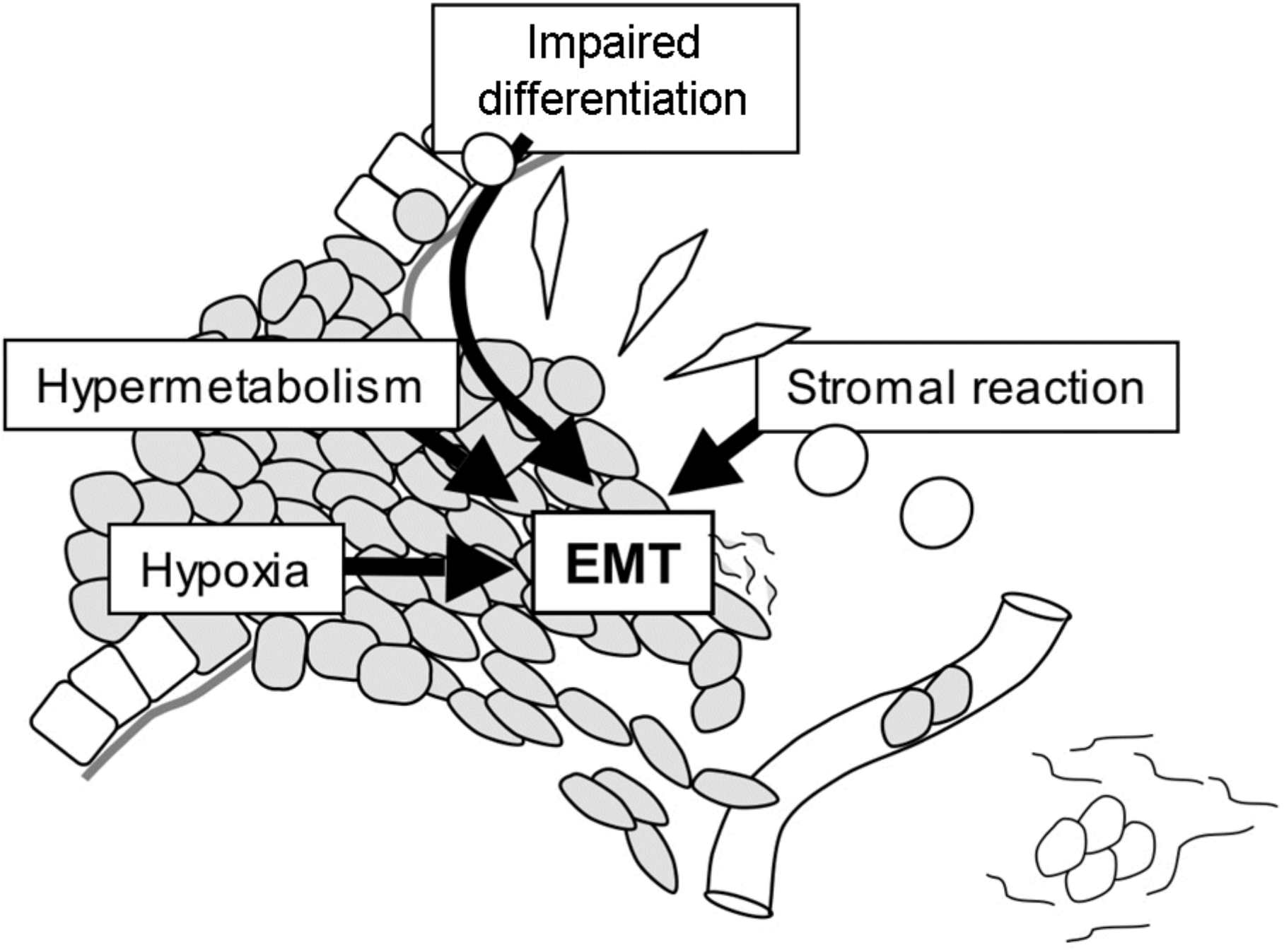
Impaired differentiation
Hypermetabolism
Stromal reaction
Hypoxia
EMT

Fig 2

Bladder Cancer	Transitional cell carcinoma	Papillary	3
		Solid	3
	Carcinosarcoma		1
Breast Cancer	Invasive ductal carcinoma		3
	Infiltrating Lobular carcinoma		2
	Carcinosarcoma		1
Colon and Rectal Cancer	Adenocarcinomas		3a
	Carcinosarcoma		1
Anal Cancer	Epidermoid carcinoma		3
Endometrial Cancer	Adenocarcinomas		3
	Carcinosarcoma		1
Ovarian cancer	Serous and Mucinous carcinoma		3
Testicular cancer	Embryonal carcinoma, teratocarcinoma		1
Gastric cancer	Diffuse gastric cancer		2
	Intestinal gastric cancer		3
Head and neck cancer	carcinoma (squamous cell carcinoma)		3
Kidney (Renal Cell) Cancer	Wilms		2
	Renal cell carcinoma		3
Liver cancer	Hepatocellular carcinoma		3
Lung Cancer	Squamous cell carcinomas		3
	Adenocarcinoma		3
	small cell lung carcinoma		3b
	Spindle-cell carcinoma		2
	Carcinosarcoma		1
Pancreatic Cancer	Adenocarcinomas		3
Prostate Cancer	Adenocarcinomas		3
	Carcinosarcoma		1
Skin Cancer	Basal cell cancer		3
	Squamous cell cancer		3
	spindle cell squamous cell		1
	Melanoma		3
Thyroid Cancer	Adenocarcinoma		3
	Anaplastic carcinomas		2
Malignant mesothelioma			1

Table 1. EMT-like phenotypes in human carcinomas. we selected three functional criteria to define "EMT-like" phenotypes in human carcinomas, ranging from partial to total EMT-like phenotype: a) state of cell polarization, b) state of cell cohesiveness and c) intermediate filament expression. Combination of these criteria helped us define the following four EMT-like phenotypes: Phenotype 0: Differentiated tumor cells with preserved epithelial structure and cell polarity, Phenotype 1: Most tumor cells characterized by cellular depolarization. However, they retain cohesive cell-cell contacts and the expression of keratins. Phenotype 2: Loss of cell-cell adhesion in most tumor cells, still expressing keratins. Phenotype 3: Loss of keratin and substantial expression of vimentin