Mesenchymal-Epithelial Transition (MET) as a Mechanism for Metastatic Colonisation in Breast Cancer

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Abstract

As yet there is no cure for metastatic breast cancer. Historically, considerable research effort has been concentrated on understanding the *processes* of metastasis, how a primary tumour locally invades and systemically disseminates using the phenotypic switching mechanism of epithelial to mesenchymal transition (EMT), however much less is understood about how metastases are then *formed*. Breast cancer metastases often look (and may even function) as "normal" breast tissue, a bizarre observation against the backdrop of the organ structure of the lung, liver, bone or brain. Mesenchymal to epithelial transition, the opposite of EMT, has been proposed as a mechanism for establishment of the metastatic neoplasm, leading to questions such as: Can MET be clearly demonstrated *in vivo*? What factors cause this phenotypic switch within the cancer cell? Are these signals/factors derived from the metastatic site (soil) or expressed by the cancer cells themselves (seed)? How do the cancer cells then grow into a detectable secondary tumour and further disseminate? And finally - Can we design and develop therapies that may combat this dissemination switch? This review aims to address these important questions by evaluating long-standing paradigms and novel emerging concepts in the field of Epithelial Mesencyhmal Plasticity (EMP).

Overview

Breast cancer is the most common malignant tumour diagnosed among women worldwide (1, 2). It is also the second leading cause of cancer-related deaths in women. In these patients, more than 90% of breast cancer-related death is caused not by the primary tumour, but by their metastases at distant sites. As a result of early diagnosis by screening, improved surgical techniques and implementation of adjuvant therapies, there is a general downward trend in the prevalence of disseminated disease in breast cancer patients (3). Although local radiation therapy and systemic adjuvant therapy reduce the incidence of metastasis by eliminating the breast cancer cells that have disseminated at the time of diagnosis, their effectiveness is far from guaranteed. More than 80% of patients receive systemic adjuvant therapy together with the initial local surgical treatment, once diagnosed with breast cancer. An estimate of the benefit of adjuvant therapy even in the most medically advanced treatment centres is a reduction in the annual odds of death ranging from 8% to 28% (4), and a reduction in the 10 year survival rate by

less than a third (5, 6). Clearly, many people are treated with these debilitating therapies to no avail.

The process of metastasis via EMT

Although uncontrolled epithelial proliferation and angiogenesis (7) are major facilitating mechanisms in metastasis, additional processes are needed for the successful establishment of a metastatic tumour (8, 9). Early in the metastatic cascade (Figure 1), cancer cells from the primary tumour acquire invasive properties and gain access to the blood or lymphatic vascular systems, which is aided by neo-angiogenesis and remodelling/destruction of the basement membrane (10-12). In the blood stream (and presumably in lymphatic vessels), intravasated circulating tumour cells (CTCs) are capable of surviving, and eventually reach 'hospitable' distant secondary sites, such as bone, lungs, brain and liver. Extravasation of CTCs at the secondary site requires recognition of and adhesion to vascular endothelial cells followed by matrix degradation (9, 13, 14). Finally, the circulating tumour cells must invade the secondary tissue to become disseminated tumour cells (DTCs), typically studied in the bone marrow. All of these processes are evidence of a more motile and plastic 'mesenchymal-like' phenotype that promotes movement from a syncitial mass and invasion through tissue (15).

The fate of DTCs at the ectopic site varies, although the vast majority of these cells do not survive even a week under experimental conditions (16, 17). The surviving cells could remain indolent as isolated DTCs or as small micrometastases. Alternatively, some DTCs could re-establish colonies to give rise to clinically overt, macroscopic secondary tumours - metastases. A daunting issue for breast cancer is the propensity for subclinical metastases to lie undetected, presumably dormant, for even over a decade before emerging.

The precise mechanisms that are involved in the transition of the subset of non-invasive tumour cells into cells with metastatic potential are still not well understood. However accumulating evidence suggests that an EMT-like process, first described in embryonic development, is one of the main mechanisms involved in breast cancer metastasis, and most likely contributes to metastases from all types of carcinomas. Somewhat consistent with its' role in normal mammary gland development (18, 19), EMT has also been shown to be responsible for converting a

fraction of non-invasive tumour cells in a solid tumour into cells with the ability to invade the basement membrane, intravasate and survive in the circulation, and extravasate at a distant secondary site (reviewed in (20-22)).

Secondary tumour formation via MET

The inefficiency of metastatic establishment has necessitated a search for an underlying mechanism that would provide for the key attributes of cell survival in an ectopic environment. A process opposite to the initial EMT at the primary tumour site, mesenchymal to epithelial transition (MET), is an evolving and relatively under-investigated mechanism that is considered to contribute substantially to the colonization of DTCs into metastatic tumours at the secondary site (23-26). Recently this well accepted 'late metastasis' concept was challenged by certain groups demonstrating that dissemination of tumour cells occurs at an early stage of primary tumour establishment (27, 28). This 'early metastasis' paradigm suggests that a fraction of primary tumour cells comprising stem cell-like characteristics with high CD44 and low CD24 ($CD44^{high}/CD24^{low}$) have the potential to depart the primary tumour site relatively early and form metastatic colonies at distant sites (29). The $CD44^{high}/CD24^{low}$ phenotype in breast cancer cell lines has been linked to EMT through the mesenchymal attributes of breast cancer stem cells, which also have dramatically enhanced malignant properties (30, 31). In either case, the disseminated cells appear to be of a mesenchymal phenotype, which is at odds with the finding of epithelial-like breast cancer nodules in the ectopic tissues (32-34).

Given the emphasis on EMT and metastatic potential, it was quite surprising that Korpal et al, 2011 (35) found recently that elevated levels of the epithelial microRNAs (miRs) 200 family in primary breast tumours already predisposed the cancer to successful metastasis, as evidenced in poorer outcomes. The miR-200 family members have been shown to promote E-cadherin re-expression via the repression of ZEB family genes and vice versa, and this mechanism has been implicated in cancer invasion and metastasis (36-40). Indeed, several 'epithelial' miRs have been implicated in promoting metastatic colonization (reviewed in (41)), supporting a role for MET. Recently, metastatic competnece in prostatic and bladder carcinoma systems was very clearly related to epithelial variants of established cell lines, rather than mesenchymal, and linked to expression of pluripotency and self renewal gene expression (42). Induction of EMT in each cell system by Snail overexpression quashed the expression of these genes, reduced tumourigenicity,

and abrogated metastatic potential. These recent studies are in sharp contrast to many studies illustrating the pro-metastatic role of EMT.

Given that epidemiological studies show that death from metastases is responsible for 90% of all human cancer related mortalities (43), and that a majority of breast cancer deaths are due to metastases rather than primary tumours (44-46), a closer examination of MET as a potential mechanism contributing to the formation of secondary breast tumours is of paramount importance, and hence the focus of this review.

E-cadherin expression in primary and secondary breast tumours

One of the key strategies in addressing this question is the assessment of the archtypical epithelial cadherin E-cadherin in metastases. These studies have been limited because, typically, metastases are not resected and thus the tissue is not available to study. Of the many EMT-related molecules, the most widely studied is the intercellular adherence protein E-cadherin (CDH1), which is currently thought to be a suppressor of tumour invasion (47-49). The functional loss or down-regulation of E-cadherin from epithelial cells is considered a hallmark of EMT (50, 51). Kowalski *et al* (2003) have reported distant metastases expressing an equal or stronger E-cadherin signal than the respective primary tumours from which they originated from (32). They saw all metastatic tumours of invasive ductal carcinoma re-expressing E-cadherin irrespective of the E-cadherin status of the primary tumours. Although not investigated, they suggested that both translational and post translational regulation of E-cadherin take place at the metastatic site in order to facilitate the establishment of secondary tumour colonies. In a another study, Saha *et al* (2007) reported re-expression of E-cadherin in bone metastasis originating from E-cadherin negative poorly differentiated primary breast carcinoma (52). In a more recent study, Chao and colleagues (2010) reported the re-expression of E-cadherin at distant metastatic tumours arising from E-cadherin low or negative primary tumours (23). They reported strong E-cadherin expression in more than 50% of liver, brain and lung metastasis originating from infiltrating ductal carcinoma of the breast (34).

Still there have been questions as to whether the few extant metastases arise from tumour cells that have undergone EMT or rare disseminated tumour cells retaining the epithelial phenotype (53). This cannot be addressed by examining human tumour specimens as all primary tumours

demonstrate phenotypic heterogeneity and the ontogeny of the metastases can only be indirectly inferred. Experimental approaches have instead begun to answer this question. First, E-cadherin downregulation was found necessary to initiate a invasion/dissemination-type response in tumor spheroid models (54). More to the point, a recent paper demonstrated that the initial spontaneous lung micrometastases from xenografts of the invasive, metastatic and mesenchymal-like MDA-MB-231 human breast cancer cell line all present re-expression of E-cadherin (23). These studies thus provide proof-of-principle that the metastatic cascade invokes E-cadherin emergence, and thus supports a MET-like phenomenon.

MET in MDA MB 468 xenograft local lymphovascular invasion

Our recent work has shed light on the need for MET in successful seeding and outgrowth of metastases from the primary site. The extant model system used a phenotypically plastic breast cancer line that responds to known tumour microenvironmental cues. The MDA-MB-468 breast cancer cell line has a modal chromosome number of 35 and was derived from a 51 year old woman with a pleural effusion in 1977 (55). It has a doubling time of 2.5-3 days (56) and demonstrates a slow migratory activity *in vitro* suggesting a low level of invasiveness (57-59). The cells are predominantly epithelial and express E-cadherin but are deficient in α-catenin (60) and lack some epithelial markers such as ZO-1 (61). Previous studies have used this cell line as a model for *in vitro* EMT after treatment with epidermal growth factor (EGF) and hypoxia (62-64). Recently this was extended to in vivo studies (65), where regional EMT could be demonstrated in the primary tumour, was evident in the CTCs by RT-PCR, and in blood vessels of both the primary tumour and lung metastases by immunohistochemistry.

Our own pilot studies have confirmed the MDA-MB-468 as a suitable model for *in vivo* EMT experimentation, and analysis of MET. Analysis of MDA-MB-468 xenografts (Figure 2) revealed that these tumours were regionally positive for vimentin *and* E-cadherin, suggesting a tumour with a so-called metastable phenotype (66, 67), a situation also noted in human breast tumour micrometastases (34). However in some regions of the tumours, as indicated with the arrow in B of Figure 2, the cells at the invasive front appeared to be arranged in thin rows in ‘Indian file’ formation, interspersed among the stromal connective tissue. These invading cells stained positive for vimentin and negative for E-cadherin, consistent with an EMT. This ‘Indian

file' appearance is typical of lobular carcinoma of the breast (68, 69), where E-cadherin loss is common (70).

Invasion of tumour cells into the neighbouring lymphovasculature was observed in association with many of the these MDA-MB-468 tumours. Two different forms of lymphovascular invasion (LVI) were observed. The majority of lymphovascular-invaded tumour cells existed as large tumour emboli, although scattered individual cells were also occasionally seen within extra-tumoural lymphovasculature. The tumour emboli consisted of tightly cohesive and considerably larger tumour cell clusters. The tumour emboli expressed E-cadherin to an extent that was noticeably higher than in the primary tumour, and also stained for cytokeratin (not shown), further confirming their epithelial nature. These observations led to speculation about the *precise* nature of the invading cells, whether these invaded cells had been mesenchymal at the time of invasion and later converted into epithelial phenotype within the vasculature, or were epithelial even at the time of invasion. However, in support the former scenario, we witnessed in some LVI a gradual transition of invaded tumour cells from mesenchymal to metastable and then to the epithelial phenotype, indicating the existence of an MET process (Figure 3).

These observations are consistent with the literature suggesting the occurrence of MET at a distant metastatic site during the formation of secondary tumours in breast cancer (23, 25, 32, 52). However, our work suggests the contribution of MET as an early event in the metastatic process. Oltean *et al* (71, 72) used FGF receptor reporter constructs to illustrate considerable plasticity in primary Dunning rat prostatic adenocarcinoma cells, and Tsuji *et al* (73) and Martorana *et al* (74) have illustrated cooperativity between epithelial and mesenchymal components in hamster oral squamous cell carcinoma and rat mammary carcinoma cellular systems, respectively. Indeed, the work of Tsuji and colleagues suggest a cooperativity model rather than plasticity *per se*, since their mesenchymal cells had an increased invasive but decreased metastatic phenotype, wheras their epithelial counterparts established lung metastases. They hypothesised that the EMT cells are responsible for leading the invasion and intravasation of epithelial cells into the blood stream to establish colonies in the secondary sites. Primary tumours of a mesenchymal nature apparently did not have sufficient plasticity to re-epithelialse at the secondary sites. This is similar to that recently reported for the bladder and prostatic

sytems described above, where cooperativity between the mesenchymal and epithelial variants for spontaneous metastasis was also demonstrated in the prostatic model both in vitro and in vivo (42). However, plasticity of the epithelial variants in vivo towards a transient mesenchymal phenotype to facilitate initial invasion away from the primary site was also demonstrated.

The expression of E-cadherin in tumour emboli has been reported in relation to inflammatory breast cancer (IBC), a distinct type of invasive breast cancer in which persistent E-cadherin is present in the primary tumour despite its highly aggressive nature (75). Therefore E-cadherin expression in local LVI is not altogether surprising, however seeing E-cadherin expressing tumour cells in the local lymphovasculature was unexpected as it usually is not seen until the stage of fruther metastasis in the target organs. These observations support the notion that E-cadherin re-expression facilitates formation of tumour cell emboli by enhancing intercellular adhesion of tumour cells. E-cadherin re-expression leads to altered receptivity towards signals from the extracellular matrix, including growth factors (reviewed in (15)).

Influence of microenvironmental factors at the secondary site which may contribute to MET

Lang and colleagues (2001) demonstrated that PC3 prostate cancer cells only underwent a MET when plated on three dimensional Matrigel, as evidenced through the formation of acinar spheroids (76), suggesting a pro-MET influence from the basement membrane substrate and/or from cellular factors expressed within the acinar spheroid microenvironment. The expression of these microenvironmental factors may be determined by the *size* of the metastases, as suggested by Kurahara et al., (2011) who demonstrated that larger (greater than 2mm) lymph node metastases from pancreatic head cancers expressed significantly higher E-cadherin compared to smaller metastases (77). Interestingly, in micrometastases of prostate cancer to the liver, the inverse was found, where the larger metastases appear to revert back to EMT (78). Some of these microenvironmental influences may be driven by the cancer cells too, since Korpal et al (35) showed that miR-200 promotes Sec23A-positive secretory vesicles, the cargo of which may regulate both autocrine and paracrine pathways to promote establishment, survival and/or growth of the macrometastases. The paracrine pathways may result in recruitment or activation of stromal cell populations.

In the clearest example of MET *in vivo* of breast cancer cells, Chao and colleagues demonstrated E-cadherin expression in lung metastases from E-cadherin *negative* MDA-MB-231 primary xenografts (23). They suggested that the re-expression of E-cadherin in metastases was influenced by the microenvironment of the metastatic site. To prove their hypothesis, they demonstrated that the E-cadherin negative MDA-MB-231 cells express E-cadherin when co-cultured with hepatocytes, a switch they had previously demonstrated in prostate cancer cells cultured under similar conditions (78, 79). E-cadherin down-regulation in cancer cells often occurs as a result of promoter methylation. Taking this into account, they postulated in the MDA-MB-231 study that loss of promoter methylation at the secondary site causes the metastatic cancer cells to re-express E-cadherin through MET. A potential demethylating factor has been identified as 1α,25-dihydroxyvitamin D3, which has been shown to promote *de novo* E-cadherin re-expression in MDA-MB-231 cells (80). Furthermore, these authors demonstrate that the receptor for this ligand, the vitamin D receptor, is positively expressed in metaplastic carcinomas of the breast.

Influence of EMT/MET states on cellular proliferative state

Several lines of evidence suggest that locally invading tumour cells undergoing an EMT proliferate *less* as they migrate *more* (81-84) (summarised in Figure 4). An early study on well-differentiated colorectal adenocarcinomas with lymph node metastasis has reported loss of the proliferative marker Ki-67 in cells along the invasive front of primary tumours, in contrast to the presence of high Ki-67 at the center of the tumours. They have observed diminished membranous E-cadherin and nuclear localized β-catenin in these Ki-67-negative cells, suggesting attenuated proliferative capacity in cells that have undergone EMT. Another study by the same group has demonstrated a higher expression of cell cycle inhibitor, $p16^{INK4A}$ (inhibitor of kinase 4) in the invasive front of well-differentiated colorectal carcinomas where β-catenin is localized in the nucleus, when compared to the $p16^{INK4A}$ negative cells with cytoplasmic β-catenin comprising the center of the tumour, confirming the hindered cell proliferation associated with EMT (84).

A direct causal link between EMT and a downregulation of proliferation may lie with the E-cadherin repressor gene set. For example, Snail1 transfected MDCK cells exhibit an arrest in cell

proliferation (85). Vega and colleagues reported that MDCK cells transfected with the transcription factor Snail underwent a complete EMT and demonstrated abolished cell proliferation resulting from diminished Cyclin D1 and D2 expression. Furthermore, it has been shown that ZEB2 mediated EMT in A431 cells led to the repression of Cyclin D1 and inhibition of cell proliferation (86). Colon cancer cells at the invasive front in which EMT is occurring, coinciding with the region where ZEB1 is expressed, display a downregulation of proliferation (84, 87, 88) (89). Therefore, it can be assumed that EMT can arrest cell proliferation though many EMT regulators such as β-catenin, Snail and ZEBs.

It can therefore be hypothesized that tumours that have undergone an MET at a secondary site become more proliferative. Elegant work by Gao and colleagues (2012) have identified bone marrow derived myeloid progenitor cells as responsible for promoting a favourable premetastatic niche (90). They identified an essential factor expressed by these cells, the chondroitin sulfate proteoglycan versican, which promoted a MET in MDA-MB-231 cells. Importantly, this factor also led to an increase in proliferation of this cell line *and* suppression of Snail1. Given the suppressive effect that Snail1 has on the cell cycle as outlined earlier, this may be the mechanism of proliferation re-activation in MDA-MB-231 cells and hence their metastases in the xenograft model, thus providing further insight into the effects of a MET in secondary breast cancers. Therefore EMT and MET may determine dormant or active states of the tumour, respectively, and allow for an indeterminate number of cycles of invasion and metastases formation.

Clinical Implications of MET-driven growth of metastases

It has been well documented that cells that have undergone EMT withstand external insults better, leading these cells to display resistance to chemotherapy and radiotherapy (91). This is particularly evident in non small cell lung cancer responses to EGFR-targeted therapies, seen both experimentally and in patients (92-94). Along similar lines, breast cancer cells remaining after neo-adjuvant treatment are enriched for EMT gene expression signatures characteristic of breast cancer stem cells (21, 95). Indeed, dramatically enhanced EMT and metastasis was demonstrated recently after vascular disruption of mammary tumours using pericyte ablation (96).

Although the exact underlying mechanism is elusive, growing evidence suggests that EMT-associated apoptosis reduction and senescence inhibition contribute largely to therapeutic resistance. Early work has revealed that EMT was responsible for rescuing serum deprived and TGF-β treated hepatocytes from apoptosis (97). It has also been reported that the EMT regulator Snail prevents apoptosis induced by serum deprivation and TNF-α treatment in MDCK cells (85). A recent study has shown the ability of EMT regulators Twist1 and Twist2 to disrupt Ras-induced senescence by inhibiting the p53 and Rb pathways (98). Arrested cell proliferation and apoptosis have been observed in breast cancer cells that have undergone EMT subsequent to prolonged TGF-β exposure (99). The EMT regulator Zeb2 has been linked as a preventer of DNA damage triggered apoptosis in bladder carcinoma cells (100). With more data accumulating, the association between EMT and reduced apoptosis is becoming more apparent.

It can be hypothesized therefore that tumours that have undergone an MET at a secondary site may be *more* susceptible to apoptotic insults, and hence may be treated more successfully with chemotherapeutic drugs. Given also that proliferation may be re-activated in MET, these secondary tumours may also be more amenable to treatment with chemotherapeutic drugs, which act on cell cycle machinery. Unfortunately this does not translate into clinical efficacy of our current chemotherapies, and this is presumed to be due to the bulk of the established metastases, which are not amenable to surgical resection/debulking the way many primary tumours are. More progress is needed to combat these larger metastases, and understanding the EMP axis may ultimately prove useful.

On the other hand, can subclinical tumour be forced to undergo a MET to facilitate therapy? This suggestion has been made, since translated to the clinic, this could reawaken the dormant, clinically silent tumour cells and render them chemoresponsive. Along these lines, clinical trials in the 1980s and early 1990s were designed to re-awaken indolent tumour deposits with growth factors to drive proliferation prior to radiation and/or chemotherapy, as had shown promise in preclinical mouse models. However these approaches did not prove useful in human tumours, and thus were stopped. More work is needed in this area to strategise around the possibilities of manipulating EMP in conjunction with chemotherapy.

An emerging possibility is that after MET, the micrometastatic tumour cells can establish cell heterotypic interactions via E-cadherin binding, as recently described (101). Such E-cadherin attachments are considered to initiate contact inhibition and suppression of proliferation (thus, the designation of E-cadherin as a tumor suppressor). As our current cancer armamentarium targets by and large only rapidly proliferating cells, this reduction in cycling would be noted as chemoresistance of this small, cryptic nodules (34).

In sum, there is ample biological precedence for viewing the MET in the metastatic site as promoting either chemosensitivity or chemoresistance. Thus, experimental model systems will be needed to settle this key question as it directly impinges on whether inducing or inhibiting MET would be beneficial in the treatment of breast cancer. Further, the question of whether the MET is stable in the metastases or if these cells show ongoing phenotypic plasticity leading to a second EMT, is also open to question. What can be said is that the view of tumor progression as a phenotypically plastic continuum rather than a relentless regression towards greater and greater degrees of dedifferentiation has opened numerous novel avenues with which to explore the biology and medicine of breast cancer metastasis.

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Figure Legends

Figure 1. The illustration elaborates the sequential EMT and MET events that are hypothesised to take place in breast cancer progression. Normal epithelial cells undergo a series of

transformational changes to become malignant. Clonal proliferation of malignant cells gives rise to invasive carcinoma. Some of these cells invade local tissues to form local metastasis while another fraction of cells undergoes EMT and intravasates into the neighbouring blood vessels. These intravasated cells may remain in the circulation as circulating tumour cells (CTCs) or extravasate at a distant site. The extravasated tumour cells may remain indolent as disseminated tumour cells (DTCs) or micrometastasis (micromets), or form macrometastasis (MacroMets) by a reverse mechanism, MET. The illustration is an adaptation from Thiery et al, 2002 (102).

Figure 2. Evidence of MET in local spread (lymphovascular invasion) of MDA MB 468 primary xenograft tumours. (A) Haematoxylin and Eosin staining indicating regions of tumor (T), peripheral stroma (S) necrosis (N) and locally metastasized tumor in the lymphovasculature (LVI); (B) To examine the EMP status, double IHC of E-cadherin and vimentin was performed using rabbit monoclonal anti-E-cadherin (clone EP700Y, Abcam, UK) and mouse monoclonal anti-vimentin (clone V9, DakoCytomation, Denmark) primary antibodies at dilutions of 1:500 and 1:100 respectively. The IHC procedure was carried out in an autostainer (BenchMark ® ULTRA, Ventana Medical Systems, Inc., USA). E-cadherin is indicated as brown colour and vimentin stained red, detected using UltraView Universal DAB (Ventana Medical Systems, Inc., USA) and UltraView Universal Alkaline Phosphatase Fast Red (Ventana Medical Systems, Inc., USA) respectively. (C) Ki-67 staining. All images were taken at magnification x200, scale bar=50μM.

Figure 3. Spectrum of MET and EMT seen in tumour emboli. A gradual transition from mesenchymal to epithelial status was observed in some established tumour emboli found within local lymphovascular spaces. These emboli consisted of regions of vimentin expressing mesenchymal cells (yellow arrow), both E-cadherin and vimentin expressing 'metastable' cells (green arrow), and predominantly E-cadherin expressing epithelial cells (white arrow). Double IHC of E-cadherin and vimentin was performed as above (Figure 2 legend). E-cadherin is indicated as brown and vimentin stained red.

Figure 4. Schematic depicting the consequence of MET on tumour growth. Mesenchymal cells which have been shed by the primary tumour may end up in the local lymphovasculature, as we

observed in the MDA MB 468 xenograft tumours, or at distant secondary sites. These locations may express factors such as versican, which drives miR-200 expression in the tumor cells to repress E-cadherin repressor genes and hence permit an MET and E-cadherin re-expression, to occur. Thus the driving 'cog' for this phenotypic change may be the expression of these microenvironmental factors, leading to the *repression* of E-cadherin repressor genes (eg. Snail1/2, Zeb1/2,Twist1/2, etc) in the tumour cells. In turn, cell cycle driving genes cyclin D1 and D2, genes that are directly repressed by Snail1 and Zeb2, may be then re-activated, restoring proliferation and tumour growth. An additional consequence of E-cadherin repressor gene repression is the re-expression of other epithelial genes such as occludin and crumbs3, and possibly the re-expression of mesenchymal genes via the tethering of β-catenin by membranous E-cadherin, preventing the activation of the Wnt pathway.

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