REVIEW

Epithelial-mesenchymal Transition and Its Role in the Pathogenesis of Colorectal Cancer

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Abstract

Epithelial-to-mesenchymal transition (EMT) is a collection of events that allows the conversion of adherent epithelial cells, tightly bound to each other within an organized tissue, into independent fibroblastic cells possessing migratory properties and the ability to invade the extracellular matrix. EMT contributes to the complex architecture of the embryo by permitting the progression of embryogenesis from a simple single-cell layer epithelium to a complex three-dimensional organism composed of both epithelial and mesenchymal cells. However, in most tissues EMT is a developmentally restricted process and fully differentiated epithelia typically maintain their epithelial phenotype. Recently, elements of EMT, specially the loss of epithelial markers and the gain of mesenchymal markers, have been observed in pathological states, including epithelial cancers. Increasing evidence has confirmed its presence in human colon during colorectal carcinogenesis. In general, chronic inflammation is considered to be one of the causes of many human cancers including colorectal cancer(CRC). Accordingly, epidemiologic and clinical studies indicate that patients affected by ulcerative colitis and Crohn's disease, the two major forms of inflammatory bowel disease, have an increased risk of developing CRC. A large body of evidence supports roles for the SMAD/STAT3 signaling pathway, the NF-kB pathway, the Ras-mitogen-activated protein kinase/Snail/Slug and microRNAs in the development of colorectal cancers via epithelial-to-mesenchymal transition. Thus, EMT appears to be closely involved in the pathogenesis of colorectal cancer, and analysis refered to it can yield novel targets for therapy.

Keywords: Epithelial-mesenchymal transition - colorectal cancer - inflammation - signaling pathway

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Introduction

Human colonic diseases are some of the most common diseases worldwide, and their incidence is increasing (Center et al., 2009). Despite welcome declines in the mortality rate over the past decade, CRC remains the second leading cause of cancer incidence and death among adult Americans. In fact, the America Cancer Society estimates that almost 150,000 new cases and 60,000 deaths will result from this disease per year. Similarly, CRC is also the second most common cause of cancer death in Europe, where comparable numbers will account for approximately 1/10 of all tumor-related deaths (Markowitz et al., 2002).

Fewer than 10% of these cases will be in individuals who have an inherited predisposition to the disease, as is the case for familial adenomatous polyposis and hereditary nonpolyposis colon cancer (Goss et al., 2000). There are also other diseases that increased the colorectal cancer risk such as Crohn's diseases and ulcerative colitis. And the close link between chronic inflammation and the development of CRC is supported by epidemiological studies. In addition, the molecular mechanisms such as oncogene activation in epithelial cells, as well as the tumor suppressor gene inactivation can also lead to the incidence of CRC (Bates et al., 2005). In the case of FAP, mutations of the APC gene result in the development of hundreds to thousands of colonic polyps, a condition that virtually assures the individual of developing CRC during their lifetime. The vast majority of CRC (approximately 70%) develop in the population as a sporadic disease (Calvert et al., 2002). Although the diversity of the etiologic and pathophysiologic factors of colonic diseases is very wide, there exists one biological process whose presence is indispensable in the progression of these conditions. This process is named as epithelial-mesenchymal transition (EMT), which has a significant role in the development of the human body. EMT was first described in early 1980s because of its pivotal role during embryonic development (Mjaatvedt et al., 1989) and later because of its implication in the physiological response to injury. In the early 1990s, EMT attracted the attention of cancer researchers after the discovery of its strong association with growth, invasion, and metastasis of cancer cells (Thompson et al., 1994). Indeed, tumor cells converts from low- to high-grade malignancy partly through EMT (Thiery et al., 2003).

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EMT is also involved in the initial step, acquisition of migratory and invasive capability of colorectal cancer, and even has an important role in tissue fibrosis (Thiery et al., 2003). As yet, increasing evidence has confirmed its presence in human colon during colorectal carcinogenesis and tumor invasion, chronic inflammation-related fibrosis and the course of mucosal healing. EMT-related molecular pathways have been extensively investigated thereafter, and various genes and molecules have been identified as important factors in EMT of cancer cells (Thiery et al., 2009). A large body of evidence supports the role for transforming growth factor-β (TGF-β) and its downstream Smad signaling, the Ras-mitogen-activated protein kinase/Snail/Slug, NF-kB pathway and microRNAs in the development of colorectal cancer via epithelial-mesenchymal transition.

Despite we have considerable knowledge refered to the molecular basis for the development of CRC, the transformation of these information into effective therapies and treatments has been limited. In this review, we will discuss EMT and its role in the development of colorectal cancer, as well as the therapeutic aspects about it.

Defining Epithelial-Mesenchymal Transition (EMT) and EMT in Carcinomas

EMT is a physiological mechanism which is present during development, and is also encountered in several pathological situations such as renal interstitial fibrosis, endometrial adhesion, and cancers (Sipos et al., 2012). Historically, epithelial and mesenchymal cells have been identified on the basis of their unique visual appearance and the morphology of the multicellular structures they create (Shook et al., 2003). The epithelial-mesenchymal transition is a process in which epithelial cells trans-differentiate and acquire an invasive mesenchymal phenotype (Techasen et al., 2012).

During EMT, the polarized and basal membrane-anchored epithelial cell undergoes a number of biochemical changes in order to acquire a mesenchymal fibroblastoid phenotype. In addition to an enhanced migration, matrix-remodeling capacity, and resistance to apoptosis, the resulting mesenchymal phenotype is characterized by several molecular and morphologic transformations. The hallmarks of EMT in vitro and in vivo include the acquisition of a spindle-like/fibroblastic morphology, the up-regulation of mesenchymal markers (i.e. N-cadherin, vimentin, α-smooth muscle actin) and extracellular matrix components (collagens α1 and α2), the down-regulation of epithelial cell surface markers and cytoskeleton components (i.e. E-cadherin, ZO-1, claudins, occludins, cytokeratins), and the up-regulation and/or nuclear translocation of specific transcription factors (i.e. Snail, Slug, ZEB1/2, Twist1/2) (Lee et al., 2006; Olmeda et al., 2007; Peinado et al., 2007;). Phenotypic markers for an EMT include an increased capacity for migration and three-dimensional invasion, as well as resistance to apoptosis. Together, these changes transform the cell from a cuboidal to a spindle shape. Finally, the cell acquires the ability to invade and move into the extracellular matrix devoid of any cell-cell contacts (Mercado-Pimentel et al., 2007). In cancer, features of EMT have been observed in breast, ovarian, colon, and esophageal cancer models (Trimboli et al., 2008).

There are evidence from human and mouse tumorigenesis models showing the involvement of EMT or partial EMT. Besides the modification of the phenotype, EMT also results in the acquisition of other properties involved in carcinoma progression, such as stemness properties (Savagner et al., 2010). In vivo and in vitro model systems have allowed the characterization of various pathways leading to EMT and EMT-like phenotypes. These pathways are referred to as EMT pathways in the present review, without assuming functional specificity. These pathways involve Akt, GSK3, Rho-GTPases, and Smad signaling pathways. Except the direct association with cancer progression, several molecules including tyrosine phosphatase Pez, CXCR447 appear to control EMT-like phenotypes and tumor metastasis indirectly (Berx et al., 2007). Down-regulation of E-cadherin is associated with cell-cell dissociation and invasion in pancreas, prostate as well as mammary gland mouse cancer models. Specific transcription factors, especially Snail, Slug, Twist, SIP1/ZEB could negatively regulate the expression of E-cadherin. At the same time, the co-expression of Snail and E-cadherin has been described in breast and colon carcinomas by several groups. In addition, the specificity of these transcriptional factors is clearly not restricted to the regulation of E-cadherin and EMT process. For example, members of the Snail family have shown to be involved in the regulation of cell motility, proliferation, differentiation and apoptotic in vivo and in cell models (Barrallo-Gimeno et al., 2005).

The tumor microenvironment is composed of inflammatory and immune cells, hypoxia, stromal, extracellular components including extracellular matrix (ECM), as well as soluble factors, and plays an important role in facilitating cancer progression and metastasis (Jing et al., 2011). 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 hyper-metabolism, glycolysis and resistance to acidosis-induced toxicity, and neo-angiogenesis. Hypoxia genes have been found to be expressed locally within solid tumors, probably contributing to tumor heterogeneity (Axelson et al., 2005). 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. In addition, Brabletz et al (Brabletz et al., 2001) compared the central areas of primary colorectal cancer and corresponding metastases, and found that nuclear β-catenin was in dedifferentiated mesenchyme-like tumor cells at the invasive front and it was localized to the membrane and cytoplasm. This study suggested that the tumor microenvironment might induce the occurrence of EMT in tumor cells. Another specific feature of the 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. Inflammatory cells is considered to play a major role in secreting activating factors, and NF-κB, a key regulator of the inflammatory response, which has been found to regulate the expression of Slug and Snail (Yang et al., 2004). Furthermore, many stem cells are found present in tumor microenvironment such as cancer stem cells (CSCs), mesenchymal stem cells (MSCs), all of which might be the inducers of EMT in tumor cells. Therefore, EMT is hypothesized to contribute to tumor progression, and indeed clinical evidence has demonstrated that regulators of EMT in cancer cells was correlated with poor clinical outcomes and tumor aggressiveness.

Roles of EMT in the Pathogenesis of Colorectal Cancer Promoted by Inflammation

Chronic inflammation has been indentified to be intimately associated with tumorigenesis include colorectal cancer (Coussens et al., 2002). Studies have confirmed Ulcerative Colitis (UC) and Crohn's Disease (CD) that two major types of inflammatory bowel diseases (IBD) with significantly higher risk to develop into colorectal cancer (Vibeke et al., 2012).

In a new population-based chort study encompassing 47347 Danish patients with IBD, 268 patients with UC and 70 patients with CD developed CRC during 30 years of observation (Jess et al., 2012). The authors concluded that the overall risk of CRC among patients with UC was comparable with that of the general population (relative risk, 1.7; 95% confidence interval, 0.95-1.21). Thirteen years after diagnosis, the CRC risk was significantly increased over background, and with longer follow-up the risk remained 50% above the risk in non-IBD individuals. On the other hand, a recent study by Herrington et al which was published in Gastroenterology assessed time changes in risk of CRC within the Kaiser Permanente Medical Care Program, a community-based health care delivery system, from 1998 to 2012. The authors indentified 29 and 53 CRC cases among CD and UC patients, respectively, corresponding to an incidence of CRC in patients with IBD which was 60% higher than in the general population (Herrinton et al., 2012). Furthermore, the incidence was found to be essentially constant over time. Chronic inflammation can lead to intestinal epithelial cells dysplasia through the induction of cellular DNA modification. In addition, DNA methylation and histone modification could also affect intestinal epithelium during development (Colotta et al., 2009). Colitis-associated cancer has been investigated in mouse models (Westbrook et al., 2010). These studies have highlighted the role of toll-like receptors (TLR) and tumor necrosis factor-α (TNF-α) in the activation of nuclear factor kB (NF-kB), which induces transcription of genes involved in tumorogenesis, including COX-2. Defect signaling via p53 may be an early event in the progression of colitis-induced dysplasia to cancer (Dirisina et al., 2011). Bataille et al. (2008) found the decreased expression of intestinal epithelial molecular marker such as E-cadherin, β-catenin, and increased expression of mesenchymal marker such as β-intergrin during the formation of fistulas in Crohn's disease. Meanwhile, they found β-catenin gradually transfer from cell membrane to cytoplasm and nucleus with the progression of EMT, and the translocation of β-catenin is considered to be the key molecular process in the development of Crohn's diseases.

There are lots of initiating factors have been found involved in cancer-related inflammation (CRI) such as NF-κB, STAT3, IL-1β, IL-6, IL-10 and TNF-α. Douglas and his colleagues (Douglas et al., 2010) inhibited the occurrence of inflammation-associated colorectal cancer using targeted inactivation of NF-κB in tumor-infiltrating cells and confirmed the close relationship between NF-κB and CRI in the initiation of colon carcinogenesis. Therefore, they provided an genetic evidence for the role of NF-κB in the progression of colorectal cancer. Lee et al. (2009) demonstrated that the activated state of NF-κB can't be maintained without STAT3 in colorectal cancer, and confirmed that STAT3 was a key factor for tumor cells proliferation and survival. Except that, they also proved the expression of c-Myc, Mcl-1, Cyclin D and Bcl-2 were regulated by STAT3. In a mouse colon tumor model, researchers found that the downstream molecules of NF-κB such as CCL2, CCL3, IL-1, IL-6 could promote the formation of inflammation-related tumors, and the missing of Tir8 in the process of NF-κB activation directly lead to the formation of colon neoplasmas. As mentioned above, TNF-α, secreted by tumor associated macrophage (TAM) could activate the signal transduction of Wnt/β-catenin through inhibiting GSK-3β, and promote the transformation from colon epithelial cells to mesenchymal, the consensus has been reached that this process is necessary for the progression of colon cancer (Douglas et al., 2010). Furthermore, Wang et al demonstrated that TNF-α induces EMT in human HCT116 cells and thereby promotes CRC invasion and metastasis. TNF-α treatment also increased the expression of transcription factor Snail, but not Slug, ZEB1 and Twist. Over-expression of Snail induced a switch from E-cadherin to N-cadherin expression in HCT116 cells, which is a characteristic of EMT. Conversely, knockdown of Snail significantly attenuated TNF-α-induced EMT in HCT116 cells, suggesting that Snail plays a crucial role in TNF-α-induced EMT. Interestingly, they found that exposure to TNF-α rapidly increased Snail protein expression and Snail nuclear localization but not mRNA level upregulation. Finally, they confirmed that TNF-α elevated Snail stability by activating AKT pathway and subsequently repressing GSK-3β activity and decreasing the association of Snail with GSK-3β. It is, AKT/GSK-3β-mediated stabilization of Snail is required for TNF-α-induced EMT in CRC cells (Wang et al., 2013). Meanwhile, the activation of NF-κB in inflammatory cells also lead to the increased expression of COX-2 and ROS, while the role of ROS can be summarized including DNA damage, DNA methylation, post-translational modification and the mutations of tumor suppressor gene (Sarkar et al., 2008). TGF-β and HIF-1, which can regulate the progression of inflammation and induce the apoptosis of tumor cells, are also the potential inducing factor that promote the transition from epithelial cells to mesenchymal in inflammatory microenvironment (López-

Novoa et al., 2009).

Grivennikov et al. (2009) have confirmed that IL-6 can augment the malignant transformation of colon epithelial cells through intracellular TGF-β signaling pathway, and IL-10 could lead to the same transformation through the same signaling pathway after the activation of STAT3 (Hoentjen et al., 2005). Except that, TGF-β also can induce the expression of transcriptional factors and transcriptional regulators in EMT such as δEF1, SIP1, Snail, thus Thuault and his colleagues concluded that TGF-β could contribute to the occurrence of EMT in colonic epithelium (Thuault et al., 2008). It is known that TNF-α is one of the most important inflammatory factors in the pathogenesis of IBD, and also plays critical role in colitis-associated colorectal cancer (CAC). It has been evidenced that TNF-α could facilitate the progression of differentiation and proliferation of colon epithelial cells depending on intracellular NF-ϰB signaling pathway, apart from that it also play critical role in suppressing apoptosis and promoting tumor invasion as well as metastasis (Greten et al., 2004).

It has been demonstrated that kinds of cytokines mentioned above participated in the development of inflammation such as TGF-β, TNF-α, NF-ϰB, and they are also critical factors for the signaling pathways of EMT. Therefore, we can conclude that EMT take part in colorectal carcinogenesis, however, the detailed mechanism of EMT in the process mentioned above need further investigation.

Role of EMT Mediated by microRNAs in Colorectal Cancer

MicroRNAs (miRNAs) are small non-coding RNAs that regulate target-mRNAs post-transcriptionally. Target recognition is based on complementary binding to the 3' untranslated region (3'UTR) of the target mRNA (Paterson et al., 2008). Translational inhibition by miRNAs is often accompanied by a reduction in target mRNA levels resulting from accelerated deadenylation of their poly (A) tail and subsequent exonucleolytic digestion (Wu et al., 2006). Accordingly, miRNAs influence numerous cellular processes including cell differentiation, proliferation, metabolism and apoptosis, in some cases by regulating one or two key target miRNAs (Wienholds et al., 2003). MiRNAs are involved in the pathogenesis of CRC, partly by regulating the expression of oncogenes and tumor suppressors and partly by functioning as oncogenes or tumor suppressors themselves (De Krijger et al., 2011). Although there are lots of studies about miRNAs involved in the pathogenesis of CRC, studies about EMT mediated by miRNAs in CRC are still less.

Recently, studies have shown that miRNAs regulate EMT through the regulation of E-cadherin and other molecules such as ZEB and vimentin (Bracken et al., 2009). For example, Cai et al investigated the expression of miRNAs in EMT and explored the effects of miRNAs on EMT in HT-29 cell line (Cai et al., 2013). In their experiment, HT-29 cells was treated with TGF-β to establish an EMT model, in which a collection of miRNAs was dynamically regulated by real-time PCR analysis. Among them, miR-21 and miR-27 were significantly upregulated, while miR-22, miR-26, miR-30, miR-181 and miR-200c were markedly downregulated. MiRNA-inhibitors were used to knockdown miRNAs in HT-29 and EMT markers were determined by qPCR to monitor the effects of miRNAs on EMT process. Results showed that miR-22 couldn't alter the expression of EMT markers, while knockdown of miR-200b could significantly increase that of epithelial markers, N-cadherin, Vimentin and Twist1 and decrease that of mesenchymal marker, E-cadherin. Their results confirmed that miRNAs are dynamically regulated in TGF-β-induced EMT of HT-29 and miR-200b was essential for EMT by suppressing the expression of ZEB1 in HT-29. A miRNA expression microarray screen was used to analyses SW480 cell clones with short hairpin RNA-mediated knockdown of ZEB1, and found the increased expression of E-cadherin and miR-141, miR-200b, miR-200c as well as decreased cell migration and invasion. Further analyses focused on miRNA-141 and miRNA-200c, which showed strongest up-regulation after knockdown of ZEB1, the results had been validated by RT-PCR and suggesting that these two miRNAs involved in the EMT of colon cancer (Burk et al., 2008). Except that, expression of miR-200c/141 cluster is regulated by DNA hypermethylation, demonstrating epigenetic regulation as a mechanism involved in the regulation of this miRNA locus (Neves R et al., 2010). Cottonham et al. (2010) revealed miR-21 and miR31 as the most elevated miRNAs after treatment of TGF-β and TNFα in regulating EMT of LIM1863, and confirmed that a high level of miR-21 or miR-31 facilitated TGF-β induced EMT of LIM1863. Unlike miR-200, which

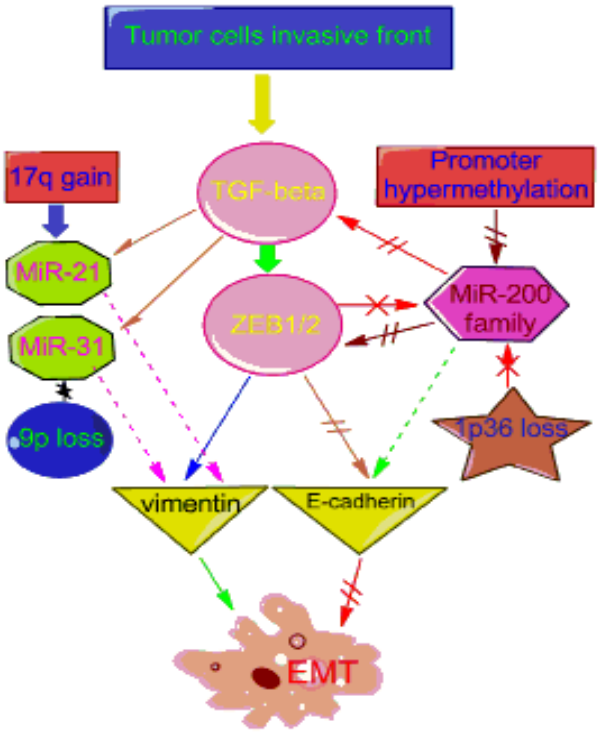

Figure 1. Regulation of Epithelial-mesenchymal Transition in CRC by miR-21, miR-31 and the miR200 Family. TGF-β: transforming growth factor beta; ZEB1/2: zinc-finger enhancer binding protein 1/2; EMT: epithelial-mesenchymal transition

suppresses an upstream master regulator of the EMT program such as ZEB1/2, miR-21 and miR-31 may impact on more downstream events such as TIAM1 resulting in a higher percentage of cells adopting a "spreading" morphology (Brabletz et al., 2011; Cottonham et al., 2010) (Figure 1). Furthermore, microRNAs such as miR-9 and miR-335 promote the metastasis of colorectal cancer by directly suppressing the levels of E-cadherin (miR-9) or SOX4 (miR-335). Except that, Sreekumar and his colleagues (Sreekumar et al., 2011) identified that regulation of E-cadherin expression was directly mediated by miR-9 via direct translational inhibition, and miR-199, miR-218 as potential regulators of N-cadherin which was thought to be a remarkable mesenchymal hallmark.

Promoter hypermethylation and associated silencing of classical tumor suppressor genes is a hallmark of human tumors. Davalos et al. (2012) showed that the 5'-CpG island hypermethylation-associated silencing of the miR-200b/200a/429 and miR-200c/141 polycistronic transcripts was a dynamic process that mediated the shifts between EMT and MET phenotypes and contributed to cancer progression and metastasis formation in tumorigenesis through laser micordisection of human primary colorectal cancer. In addition, they found that the hypermethylation of miR-200 and up-regulation of ZEB1/ZEB2 were associated with down-regulation in CDH1, CRB3 and LGL2, and they demonstrated that miR-200 hypermethylation-associated inactivation in TGF-β induced EMT was accompanied by increased expression of ZEB1, loss of E-cadherin and the acquisition of a spindle-like shape, a mesenchymal phenotype marked by Vimentin (Kenney et al., 2011). De Krijger and his colleagues (De Krijger et al., 2011) confirmed that in human CRC, a significant subset (36%) of primary tumors showed decreased expression of miR-34a, partially due to the presence of TP53 mutations, another mechanism refer to the down-regulation of miR-34a was the loss of chromosome 1p36 (the location of miR-34a), which was observed in 50% of primary CRCs, 33% of the local recurrences, and 64% of the metastases. Alternatively, epigenetic inactivation of miR-34 family (consisting of miR-34a, miR-34b and miR-34c) by promoter hypermethylation is observed in the majority of cancer cell lines and primary CRCs.

Signaling Pathways involved in the Progression of EMT

During the progression of EMT, there are several signaling pathways involved in and leading to the aberrant expression of E-cadherin, Vimentin, cell phenotypic changes, degradation of the basement membrane, and so on (Figure 2).

The SMAD/STAT3 pathway

Thus far, the Smad pathway was the most-studied TGF-β downstream signaling cascade. The Smad protein superfamily is composed of the receptor-regulated Smad 1, 2, 3, 5 and 8; the co-Smad Smad4; and the inhibitory Smad6 and 7. Upon TGF-β binding to its receptor, Smad 2 and 3 are activated through phosphorylation of their SSXS C-terminal motif. Subsequently, they form homo- and heteromeric complexes with Smad4 and are translocated to the nucleus to ultimately regulate the transcription of targets (Conidi et al., 2013). Contradictory in vitro data made the role of Smads in EMT controversial until in vivo targeting of Smad4 shed light on its pivotal role in pancreatic cancer EMT. Blockade of EMT in Smad4-deficient pancreatic tumors is characterized by epithelial morphology and organization, persistence of epithelial markers, and lack of mesenchymal markers such as vimentin in tumor cells (Carla et al., 2010). Nevertheless, Smads seem to interplay with other TGF-β signaling pathways to induce EMT, and could even participated in negative regulation of the process. Using isogenically matched cancer cells, Zhao et al. demonstrated that although Smad4 is necessary for TGF-β-mediated down-regulation of E-cadherin and β-catenin and for vimentin induction, Smad4 wild-type cells present reduced invasion and metastasis in an orthotopic model of pancreatic cancer (Zhao et al., 2008). This observation may be explained by TGF-β-mediated inhibition of STAT3 phosphorylation in Smad4 wild-type cells. Therefore, STAT3 activation is required for invasiveness of pancreatic cancer cells and Smad4 is a negative regulator of this STAT3 EMT-associated function. Conversely, loss of Smad4 leads to aberrant activation of STAT3 and may contribute to the switch of TGF-β from a tumor-suppressive to a tumor-promoting pathway in colorectal cancer (Ono et al., 2012). It is interesting to note that the variety of cell responses exhibited n response to TGF-β are governed primarily by the cell type-specific expression of various Smad2/3 interacting transcription factors (Galliher et al., 2006).

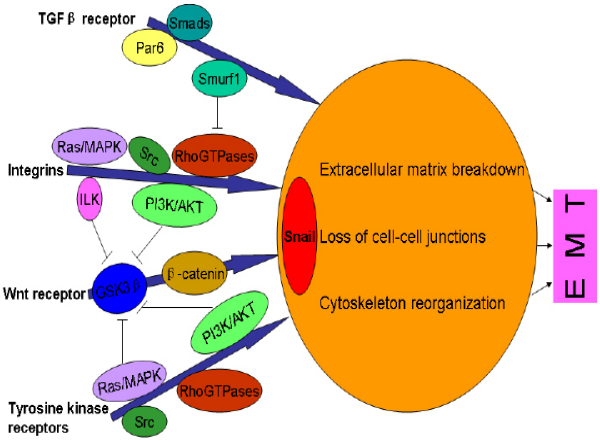

Figure 2. Simplified Diagram for the Signal Pathways Involved in EMT. EMT occurs by hyperactivation of one pathway or, more probably, by simultaneous activation of more pathways, which either leads to Snail-mediated down-regulation of the E-cadherin gene, as Ras-Raf-MEK-MAPK, PI3K-Akt, TGF-β-Smads, ILK-Akt, and Wnt-β-catenin signaling do, or directly affects cell adhesion and/or the cytoskeletal dynamics, as accomplished by Src,TGFβ-Par6-Smurf1 and Rho GTPase. Moreover, ILK and Akt stabilize β-catenin by phosphorylating GSK3β, and thereby they can affect the expression of cell-cycle activating molecules stimulated by the β-catenin signaling such as Myc and cyclin D1. In addition, Akt promotes cell survival by inhibiting pro-apoptotic proteins. Therefore, EMT could be coupled to changes in proliferation and/or to survival-promoting responses that might be required to ensure the capability to survive as single cells in a foreign environment

In addition, TGF-β typically represses NF-kB activity in normal epithelial cells, but readily activates this transcription factor in their malignant counterparts (Neil et al., 2008). More recently, TGF-β has been shown to activate a number of protein tyrosine kinases (PTKs), including FAK, Src and Abl, which results in the inappropriate amplification of noncanonical TGF-β signaling in mesenchymal or dedifferentiated epithelial cells. Moreover, imbalances in the activation status of canonical and noncanonical TGF-β signaling systems may very well underlie the ability of TGF-β to induce EMT in normal and malignant cells (Michael et al., 2009).

Ras pathway

Several evidences indicate the implication of the Ras/ERK1/2 pathways in the mesenchymal transformation of cancer cells. First of all, oncogenic Ras or ERK overexpression leads to mesenchymal transformation of MCF-10A breast cancer cells (Shin et al., 2010). The interplay between Ras and ERK2 was established by the abrogation of Ras-induced EMT after ERK2 shRNA-mediated knockdown. In pancreatic adenocarcinoma, there is a positive correlation between EMT and the activation of ERK in cancer cells, with poor survival of patients (Javle et al. 2007). Ras proteins act as molecular switches that cycle between active GTP-bound and inactive GDP-bound forms and function as essential components of signal transduction pathways regulating cell growth. Indeed, upon activation by growth factor-stimulated receptors, activated Ras complex with and promotes Raf kinases, which in turn active MAPK kinases (MEK1 and MEK2), resulting in activation of extracellular signal-regulated kinases (ERK1 and ERK2) (Cuevas et al., 2005). Activated ERKs then translocate into the nucleus where they phosphorylate and activate downstream nuclear transcription factors, such as Elk-1, ATF-2 and ETS1/2, and finally resulting in immediate-early gene induction.

The involvement of these downstream effectors in intestinal tumorigenesis is also supported by a number of experimental results. First, mutations of BRAF, a member of the Raf family, is associated with increased kinase activity and have been found in 9-11% of colorectal cancers. Second, it has been demonstrated that MEK is phosphorylated and activated in 30-40% of adenomas and in 76% of colorectal cancers. Third, colorectal cancers exhibit high frequencies of ERK activation and studies have reported that ERK1/2 activity is elevated in intestinal tumors. Fourth, blockade of MEK/ERK suppresses the growth of colon tumors in vivo, suggesting that ERK involved in the progression of intestinal tumor proliferation. The analyses of Snail 1 and Snail 2 promoter revealed that Egr-1 as well as Fra-1, an AP-1 protein (downstream effector molecule of ERK/MAPK, the expression of proteins forming AP-1 in colon cells named c-jun, c-fos and Fra-1), are responsible for MEK1-induced expression of Snail 1 and Snail 2, respectively (Lemieux et al., 2009). In addition to switching on the MAPK cascade, Ras can also signal through PI3K and RhoGTPases, and can synergize with transforming growth factor-β (TGF-β) in inducing EMT (Guarino et al., 2007). The process mentioned above could result in the increased expression of Vimentin and decreased expression of E-cadherin, and demonstrate that the expression of activated MEK1 is sufficient for EMT of colon epithelial cells.

NF-kB Activation and Epithelial-Mesenchymal Transition

Studies have shown that primary cancers and their corresponding metastatic tumors exhibited mixed epithelial-mesenchymal phenotype. Cells in the tumor center remain positive for the expression of E-cadherin and cytoplasmic β-catenin, and the tumor cells in the periphery display loss of surface E-cadherin and upregulation of vimentin, the typical characteristics of EMT phenotype (Koay et al., 2012).

Importantly, increasing evidence has shown that NF-kB activation is required for the induction and maintenance of EMT. It has been found that NF-kB suppresses the expression of epithelial specific genes, E-cadherin and desmoplakin, and induces the expression of the mesenchymal specific gene vimentin (Tang et al., 2012). Repression of E-cadherin expression by the transcription factor Snail is a central event during the loss of epithelial phenotype. NF-kB has been found to induce the expression of Snail, leading to the downregulation of E-cadherin (Wu et al., 2011). NF-kB also upregulates transcription factor ZEB1 and ZEB2, resulting in the inhibition of E-cadherin expression during EMT (Chua et al., 2007). Furthermore, studies have shown that NF-kB could induce Bcl-2 expression and promote invasion of breast cancer cells, which are associated with EMT (Wang et al., 2007). Recently, Kong et al have found that overexpression of PDGF-D could activate NF-kB and thereby upregulates Bcl-2, which may contribute to EMT phenotype and invasive behavior of PC-3 prostate cancer cells (Kong et al., 2008). In addition, gene expression profile analysis has shown that the genes involved in EMT and NF-kB signaling deregulation are the most prominent molecular characteristics of the high-risk head and neck squamous cell carcinoma, suggesting that NF-kB signaling plays important roles in EMT (Chuang et al., 2006). Therefore, inhibition of NF-kB could stop the progression, invasion and metastasis of cancer in part due to deregulation of the processes of EMT.

Therapeutic Aspects of EMT in Colorectal Cancer

Inhibition of EMT would be an ideal choice for the treatment of CRC, as well as colonic fibrosis development on the basis of chronic inflammation.

TGF-β has been confirmed involved in the regulation of cell proliferation, differentiation, and apoptosis and associated with EMT. Therefore, the inhibition of TGF-β pathway is an attractive strategy for the treatment of colorectal cancer. Previous study have suggested that protein-bound polysaccharide (PSK) modulated the biological activity of TGF-β1 and β2 by binding to their active forms (Matsunaga et al., 1998). Yoshihiro et al. (2012) recently demonstrated the inhibitory effect

of PSK on the TGF-β pathway and TGF-β-induced EMT. In their study, they found PSK inhibited the Smad pathway, the major regulator of TGF-β signaling, by suppressing Smad2 protein phosphorylation. Protein-bound polysaccharide also decreased the levels of both c-Jun and phosphorylated c-Jun, which were increased by TGF-β1 treatment, suggesting that the effect of PSK involves in the MAPK pathway. Therefore, the present results show that the inhibitory effect of PSK on TGF-β pathway and indicate that PSK could be a promising new agent for the treatment of diseases, such as colorectal cancer associated with alterations in TGF-β signaling. In addition, several randomized clinical trails have shown that PSK has anticancer potential in adjuvant cancer therapy, with positive results in the treatment of breast, gastric, and colorectal cancers (Sakamoto et al., 2006).

Activation of phosphatidylinositol 3-kinase (PI3K)/Akt signaling through activating mutations in PI3KCA or loss of PTEN is associated with the progression of CRC (Roy et al., 2002). Gulhati et al. (2011) have previously shown that the mammalian target of rapamycin (mTOR) kinase, a downstream effector of PI3K/Akt signaling, could regulate the tumorigenesis of CRC. Their recent findings support a role for elevated mTORC1 and mTORC2 activity in regulating EMT and metastasis of CRC. Consistent with their findings, a recent study found that the cytokine TGFβ could induce the activation of mTOR signaling. The inhibition of mTOR signaling using rapamycin inhibited the increase in protein synthesis and cell size, while inhibited cell migration and invasion associated with TGF-β-induced EMT (Lamouille et al., 2007). Taken together, previous results showed that both mTORC1 and mTORC2 could contribute to CRC tumorigenesis, it may be hypothesized that the inherent redundancy in functions of both complexes may allow mTORC2 to compensate for the loss of the activity of mTORC1 upon rapamycin treatment, thereby leading to rapamycin resistance. In addition, recent studies have shown that IGF-1, EGF, and TGFβ are critical mediators of EMT which could activate mTOR signaling and inhibit the function of mTOR using rapamycin (Zhang et al., 2010). Based on these data, researchers provide the rationale for including mTOR kinase inhibitors targeting the ATP binding pocket, which inhibit both mTORC1 and mTORC2 more completely, as part of the therapeutic regimen for treating CRC patients.

Protein kinase CK2 is a highly conserved, ubiquitous protein serine/threonine kinase that phosphorylates many substrates and has a global role in numerous biological and pathological processes. Zou et al. (2011) have assessed the expression of CK2α in colorectal cancer, adenoma, and normal colorectal epithelium and found the expression of CK2α was much higher in CRC than in adenoma, they also found that suppression of CK2α by siRNA or the CK2α activity inhibitor emodin inhibited the proliferation of CRC cells, caused G0/G1 phase arrest, elevated the expression of p53/p21 and decreased the expression of C-myc. They also found that knockdown of CK2α suppressed cell motility and invasion. Significantly, the inhibition of CK2α resulted in the transactivation of β-catenin, decreased the expression of Vimentin and transcription factors Snail 1 as well as Smad2/3, and increased the expression of E-cadherin, suggesting that CK2α may be involved in the progression from adenoma to CRC, and regulates the epithelial-mesenchymal transition (EMT) process in cancer cells. Previous study has proved that the progression from normal intestinal mucosa to adenoma (adenomatous mucosa) and finally to adenocarcinoma in CRC is closely correlated with the EMT process and changes in the expression of series of genes, such as E-cadherin, Vimentin, and β-catenin (Chen et al., 2008). Therefore, inhibition of CK2α may serve as a promising molecular target for the treatment of human CRC.

The EMT itself is not a cell-autonomous process and requires initiating signals to drive the transition, in particular, TGF-β has been implicated as a key inducer of this event. Bates et al. (2005) used a colon carcinoma model to analyze integrin dynamics as a function of EMT and found that the transition results in the regulation of the integrin αvβ6, a receptor for the ECM protein fibronectin and tenascin. αvβ6 is an activator of latent TGF-β, and it was shown that post-EMT cells in their system indeed acquired the capacity to activate latent TGF-β. In addition, while this effect was completely attributable to the increase in the levels of αvβ6 intergrin, these cells also displayed an autocrine production and secretion of the cytokine as a consequence of EMT (Bates et al., 2005). Thus, in the context of an in situ tumor, they predicted that autocrine production of TGF-β would provide the means to sustain and stabilize the EMT process. The concomitant expression of αvβ6 would in turn provide an activation mechanism allowing presentation of active cytokine to adjacent cells, resulting in a perpetuation of the EMT process and the creation of a localized microenvironment more amenable to progression. The potential implication of these findings is important because the integrin αvβ6 can turn to an attractive new candidate as a therapeutic target for metastatic colon carcinoma.

Several miRNAs have been reported to influence tumorigenesis by down regulating PTEN translation, including miR-17-92, miR-19a, miR-26a, miR106b, miR-214 and miR21 in colorectal cancer (Sreekumar et al., 2011). Other than PTEN, the mRNAs of proteins in the axin/APC/β-catenin/GSK3β complex are also targeted by miRNAs. miR-200a takes part in a negative feedback loop with the ZEB family of transcription factors, thus modulating the cadherin complex, and is also involved in direct translational repression of β-catenin mRNA in CRC (Saydam et al., 2009). miRNAs targeted to mRNAs, encoding stem cell signaling components or EMT regulators, are also potent drug targets. MiRNAs including proliferative, anti-apoptotic, pro-angiogenic, or pro-metastatic effects on tumor cells could be downregulated for cancer therapy, while those with proapoptotic, anti-angiogenic, or anti-metastatic effects could be applied for synthetic miRNA (Katoh, 2008). RNA aptamers binding to the extracellular region of Pacthed1 could be utilized for drug delivery to cancer cells with Hedgehog signaling activation. RNA aptamers binding to the cytoplasmic region of Smoothened and those binding to Fused or GLI1 could be utilized as Hedgehog signaling inhibitors

(Varnat et al., 2009). Peptide mimetics, resembling Wnt and fibroblast growth factor family members, have been developed (Li et al., 2008). Because Wnt5A is involved in the non-canonical signaling cascade for the induction of EMT partly through Snail 1 upregulation, a Wnt5A mimetic is able to suppress invasion and metastasis of cancer cells (Dissanayake et al., 2007). In addition, when specific miRNAs involved in the process of metastasis are identified, therapeutic strategies can be developed that aim at the silencing of oncogenes or up-regulating tumor suppressor genes. In order to silence an oncogene, reintroducing or over-expressing the miRNA that targets the oncogene is needed. A second option for cancer treatment could be the down-regulation of miRNAs that suppress the function of tumor suppressor genes. This may be achieved by the introduction of antisense oligonucleotides or antagomirs, which are synthetic analogues of miRNAs that bind irreversibly to and inhibit the function of the miRNA of interest (Vidic et al., 2010). On the other hand, great care should be taken before clinical application of these technologies, as the miRNA and siRNA off-target effects are serious problems.

Conclusions

According to our current knowledge, EMT is a highly significant biological event, not just in physiological, but in pathological circumstances. In spite of rapid advances in our understanding of the molecular genetics of CRC, the burden of disease remains high and the outlook for patients with advanced cancer is still poor. Therefore, the emerging understanding of the molecular pathways underlying EMT could provide novel opportunities in the treatment of colorectal cancer patients by preventing this aspect of invasion. As EMT is triggered by E-cadherin repressors, targeting Snail members represents a potential tool for contrasting EMT and invasion. Similarly, molecules that able to modulate Snail's stability and sub-cellular localization such as GSK-3β and PAK, as well as extracellular factors including TGFβ and metalloproteinases could be used in the near future as targets for preventing EMT.

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