

Emerging Role of Transcription Factors in Epithelial Mesenchymal Transition Attributing to Metastasis of Colorectal Cancer

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Carol Chandy¹ and Radhashree Maitra^{1*}

¹Department of Oncology, Albert Einstein College of Medicine & Montefiore Medical Center, New York, USA

Abstract

Epithelial Mesenchymal Transition (EMT) is one of the major phenomenon that cancer cells utilize to lose their identity and travel to remote sites thus causing metastatic tumors. Transcription factors plays determining roles not only in the initiation of tumorigenesis, but also during intravasation, extravasation and establishment of distant metastatic tumors. Epithelial cells of the gut mucosa are polar in nature with differential expression of surface markers at the apex and the base. The loss of polarity is the first step towards EMT. This is followed by rearrangement of the cytoskeletal architecture and loss of integrity to the basement membrane. Unbound cells have the propensity to over grow and become hyperplastic. The process continues to develop dysplastic modifications and finally the neoplastic transition occurs. These neoplastic cells then further dedifferentiate to become the mesenchymal cells. This complete series of events are critically controlled by different transcriptional factors. Herein we focused on the newly emerging transcription factors that contribute to the epithelial mesenchymal transition in colorectal cancer. We have selectively studied several transcription factors exploring their newly determined role in EMT.

Keywords: Transcription factors; Colorectal cancer, Epithelial mesenchymal transition; FOXC2; E- cadherin and metastasis

Introduction

Colorectal cancer (CRC) known to be second most common visceral malignant neoplasm in United States with over 200,000 cases per year [1]. Throughout the years, treatment options for colorectal cancer have improved dramatically due to the immense research being conducted worldwide. These studies have guided the medical professionals to have better insight of the disease at molecular levels leading to better targeted therapeutics. Cancer becomes lethal when cells metastasize, a multifactorial process of malignant cells spreading throughout the body from the primary tumor site. Halting the metastatic progression has been the predominant area of focus that has proven to increase the survival rate of patients with CRC. The activation and deactivation of cell signaling pathways that induce or dissuade the transcription of proto-oncogenes and tumor suppressor genes are being constantly explored by researchers. Among the many identified modulators, those which play pivotal role in transcriptional and translational regulations has been dissected meticulously. The review focuses on the role of different regulatory factors promoting epithelial mesenchymal transition (EMT) which leads to metastasis of CRC.

Role of EGF and VEGF in EMT

The role of epidermal growth factors (EGF) and vascular endothelial growth factors (VEGF) in modulating and promoting metastasis has been widely examined. Matrix metalloproteinases (MMP) secreted by EGF receptors are known to but not limited to the degradation of extracellular matrix (ECM) of basement membranes allowing cancer cells to squeeze through tight junctions between endothelial cells of blood or lymphatic vessels [2]. VEGF, a proangiogenic factor initiates angiogenesis. Compendiously, when additional vasculature is needed, tumor cells excrete proteins into the extracellular regions stimulating the growth of blood vessels towards the tumor region [2]. VEGF family of receptors located on the endothelial cells of blood vessels detect the proteins secreted by the tumor cells causing them to proliferate via the activation of extracellular kinases and MAPK signal-transduction pathways [3]. The activation of these kinases induces MMPs, urokinase plasminogen activator system (uPA), and its' receptor uPAR

*Corresponding Author: Radhashree Maitra, Department of Medicine, Albert Einstein College of Medicine, New York, USA, Tel: (718) 430-4143, E-mail: rmaitra@montefiore.org

which motivates vessels to be more permeable and motile. The degradation of the extracellular matrix by MMPs allow loosened endothelial cells to maneuver and branch towards the tumor site to serve the purpose of additional vasculature [3]. The epithelial mesenchymal transition is a sequence of mechanisms where baso-apical polarity and cell to cell adhesion is fractured to allow migration and invasion of the tumor cells which in turn undergo transformation into poorly differentiated mesenchymal stem cells [4]. EMT is currently being investigated to understand metastasis to its core. E-cadherins are major calcium dependent transmembrane proteins that facilitates the continuity of cell-cell adhesion [3]. Tumor cells that are attached to epithelium via the E-cadherins are disintegrated prior to metastasis. Cancer cells utilize colony stimulating factors (CSF) namely Granulocyte CSF and Macrophage CSF to recruit macrophages that secrete EGF, promoting the fracture of cell-cell adhesion, where this process is continued in a positive feedback loop [5]. Cathepsin B proteins encoded by CTSB gene are also released persuading the intracellular proteolysis, accelerating the cleaving of E-cadherin attachments [6]. Macrophages releases Tumor necrosis factor alpha (TNF α) and EGF to stop E-cadherin production and initiate N-cadherin (mesenchymal factor) production. E-cadherin suppresses the invasion in epithelial cells whereas N-cadherin is active when E-cadherin is downregulated favoring EMT [7].

Chemo attractant factors such as Hepatocyte growth factor (HGF), often overexpressed in colorectal cancer attracts tumor cells along a chemical gradient causing actin rearrangements through Ras like GTPases [8]. The N-cadherin then allows the cancer cells to interact with other stromal cells, later leading to hematogenous or lymphatic spread [7]. The role of transcriptional factors and regulators that conjointly collaborate to achieve EMT is the first step in understanding the progression of metastasis. EMT can be triggered by a plethora of growth-factors, and

prominent signaling pathways like Transforming growth factor beta (TGF- β), HGF, EGF, Wnt, and Hedgehog which have been shown to initiate and promote EMT in cancer, organ fibrosis, and embryonic development [9]. Down-regulated epithelial markers (E-cadherin, plakoglobin and desmoplakin), upregulated mesenchymal markers (Vimentin, N-cadherin and β -smooth muscle actin) and increased expression of transcription factors such as Snail, Slug, Twist, zinc finger E-box binding homeobox 1 (ZEB1), ZEB2, and/or E47, which can bind to E-cadherin promoter and inhibits its transcriptional activity and expression are the molecular hallmarks during EMT [10]. Hypoxia Inducible factor (HIF- α) has a wide recognition in the involvement of metastatic process, treatment and poor prognosis [10].

Role of transcriptional factor FOXC2 in promoting colorectal metastasis

FOXC2 belonging in Fork head family of transcription factors is an important molecular factor that has a pivotal role in lymphatic vasculature remodeling thus also playing a crucial role in lymph node metastasis in colorectal cancer [1]. It is known that the down regulation of FOXC2 lead to apoptosis of cancer cells [1]. The interaction with PIN1, ERK1/2 and PP2A by FOXC2 motivates phosphorylation that regulates FOXC2 transcriptional program in primary lymphatic endothelial cells [11]. Selective inhibition of FOXC2 recruitment to chromatin has shown changes in lymphatic endothelial cells through genome wide analysis [11]. ShRNA mediated suppression of FOXC2 in mammary epithelial cell lines has exhibited to undergo EMT via the ectopic expression of SNAIL, TWIST or TGF- β (Figure 1) [12]. Ectopic expression of EMT in mammary epithelial cancer cell lines also showed the sufficiency of FOXC2 to induce robust EMT and stem cell like properties [12]. This was observed by the reduction in the expression of epithelial specific E-cadherin in conjunction to the increased expression of

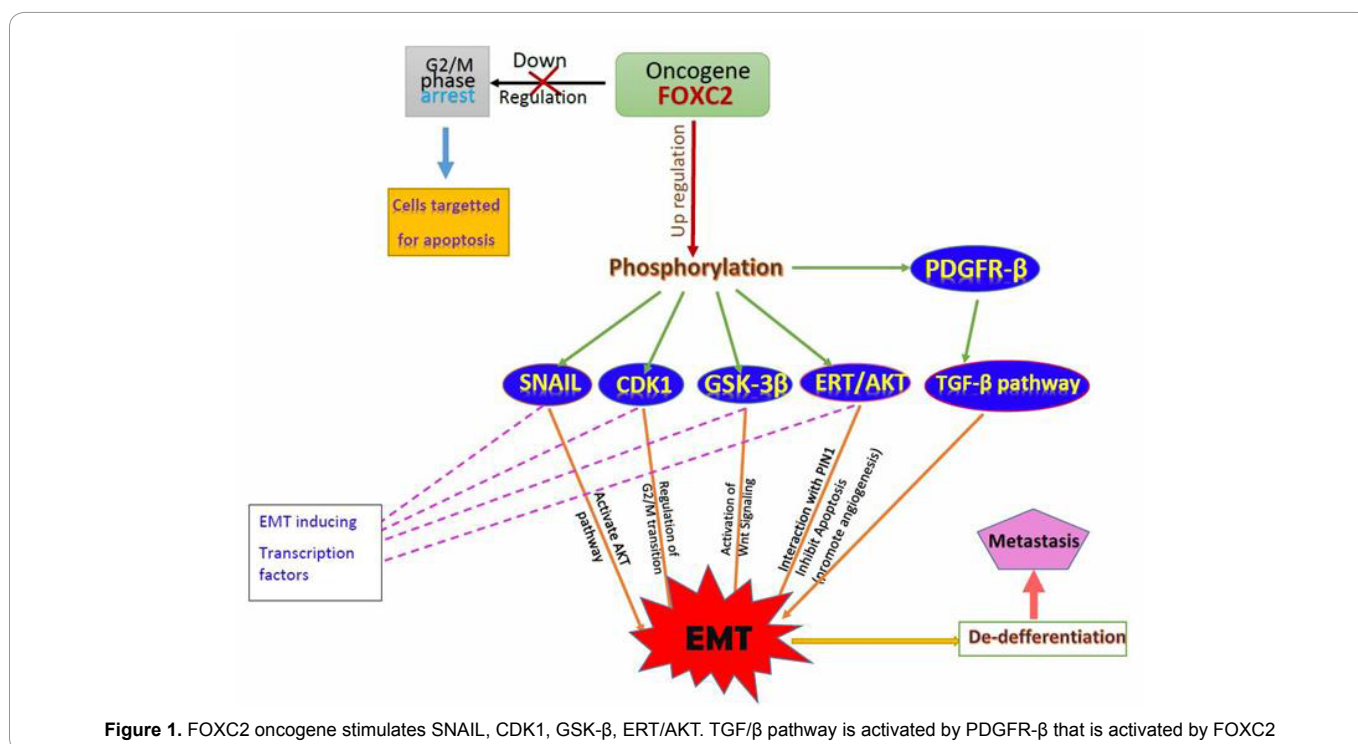


Figure 1. FOXC2 oncogene stimulates SNAIL, CDK1, GSK- β , ERT/AKT. TGF/ β pathway is activated by PDGFR- β that is activated by FOXC2

mesenchymal markers (Vimentin, fibronectin and N-cadherin) at the mRNA and protein levels [12]. FOXC2 could also be a potential transcriptional regulator of PDGFR- β (Platelet Derived Growth Factor Receptor beta) expression due to the substantial decrease of PDGFR- β expression upon the suppression of FOXC2 [12]. PDGFR- β expression in CRC was strongly correlated with both TGF- β signaling and with key EMT-driving transcription factors promoting liver metastasis by the formation of Mesenchymal-like colorectal tumor cells [13]. Inhibition of GSK-3 β resulted in the decrease of nuclear FOXC2 and increase in cytoplasmic FOXC2 [14]. FOXC2 expression regulates the G2/M phase transition and is post-transcriptionally regulated in a cell cycle dependent manner partially by PLK1 (Polo Like Kinase 1) [14]. Inhibition of PLK1 causes a decrease in FOXC2 protein levels. FOXC2 was abundant in late G2/attached cells compared to mitotic phase [14]. The activity of PLK1 is essential for FOXC2 stability in G2 [14]. CDK1 (Cyclin dependent kinase 1) is the master regulator of G2/M transition that is downregulated by the phosphorylation of FOXC2 [14]. FOXC2 activity is regulated by phosphorylation and mutating the key phosphorylation sites in FOXC2 inhibit chromatin binding, causing differences in the gene expression profile of many mitotic proteins or G2/M regulators [14]. The expression of FOXC2 gene significantly differed between N1 and N2 lymph node metastatic CRC patients compared to CRC patients without lymph node metastasis [15]. The lymph node-positive group displayed more than twofold level of FOXC2 messenger RNA expression than patients in the lymph node-negative group [15]. This confirms the importance of FOXC2 gene acting as a target to predict the metastasis to lymph nodes in CRC. FOXC2 also has a role in CRC by the downregulation of FOXC2 gene enhancing apoptosis by 5-fluorouracil, a chemotherapeutic drug, through activation of MAPK and AKT pathways [16]. The focus of this study in contrast to previous study was more directed towards the reason for the resistance to 5-fluorouracil correlated with abnormal expression of FOXC2 [16]. Ablation of FOXC2 expression led the human colon, cancer cell line HCT 116 became sensitive to 5-fluorouracil [16]. By analyzing p-AKT and total AKT levels, it is confirmed that the MAPK and P13K/AKT pathways are essential for cancer cells to be sensitive to 5-fluorouracil [16]. While many Forkhead transcription factors have been implicated in the regulation of the cell cycle, the fact that FOXC2 expression is restricted to cancer cells with stem cell properties, as well as its central role in the regulation of EMT and metastasis, attest to its potential utility as a therapeutic target.

Role of Forkhead Box Gene and ROCK2 gene in EMT

Forkhead Box M1 (FOXM1) is a proto oncogene that induces mitosis and is considered as a proliferation specific transcription regulator [17]. Other functions of this gene include invasiveness and angiogenesis that are major events in the process of metastasis [17]. FOXM1 regulates the expression of MMPs and VEGFs. It also upregulates the expression of lysyl oxidase, ZEB1/2 and SLUG, as a result leading to reduction of the expression of E-cadherin which is why FOXM1 is an important regulator of EMT and metastasis [17]. The study emphasizes on how the ROCK's (Rho associated coiled-coil protein kinase2) activation works parallel for the achievement of the regulation FOXM1D, an isoform of FOXM1 in regulating cancer cell metastasis [17]. E-cadherin staining was remarkably reduced on the contrary to the significant increase of mesenchymal markers N-cadherin and vimentin in over expressed FOXM1D CRC cell lines [17].

Moreover, over-expressed FOXM1D cancer cell lines displayed more extended and elongated shape than control cells. Although the isoforms, FOXM1B and FOXM1C over-expressed cells showed remarkable migration and invasion, FOXM1D showcased more potential in comparison [17]. In in vivo study conducted, FOXM1D-overexpressed mice promoted inguinal lymph node metastasis compared to the control ones. On the other hand, FOXM1D knockdown in other CRC cell lines showed significant reduction in metastases over the control [17]. FOXM1D-overexpressed mice showed multiple metastases in the liver and abdominal cavity. Lastly, through co-immunoprecipitation (co-IP) coupled mass spectrometric analysis, ROCK2 was identified as one of the main FOXM1D binding protein [17]. It was demonstrated that only FOXM1D interacted with ROCK2 compared to its' three isoforms. Both ROCK1 and ROCK 2 interacted with FOXM1D influencing its role in EMT and metastasis. FOXM1D employs a unique mechanism to promoting EMT and metastasis by activating ROCK2 together with GTPases, thus leading to cell detachment, motility and invasion by cytoskeletal rearrangement [17]. In a nutshell, transcriptional factor FOXM1D could be potential biomarker or therapeutic target in colorectal cancer metastasis [17].

Transcription factor YAP1 and CDK in relation to KRAS proto-oncogene

Metastases in colorectal cancer are mainly hematogenous or lymphatic [1]. The profusion of lymphatic and blood vessels surrounding every part of the colon is a good opportunity for cancer cells to percolate into and metastasize. It is proposed that hypoxia of the tumor cells is the ultimate reason for the initiation of metastasis. About 90% of the patients diagnosed with CRC have higher mortality rate due to metastasis [18]. KRAS mutation has been documented in 40-45% of patients with CRC [19]. Activating mutations in the KRAS gene cause the constitutive activation of Ras GTPase, which leads to the over-activation of downstream Raf/Erf/Map kinase and other signaling pathways, resulting in cell transformation and tumorigenesis [20]. A recent study was conducted with 494 patients, of which 202 (41%) had tumors with KRAS mutation. The time to lung metastasis (TTLM) was observed to be shorter in patients with KRAS mutation with a twofold greater odds of developing lung metastasis in patients with liver-limited metastatic CRC [20]. Furthermore among the 275 patients who initially had liver as the only site of metastatic disease, patients that had KRAS mutant allele were twofold more prone to develop lung metastasis [20].

The activation of pleiotropic signals is triggered by KRAS oncogene that triggers the initiation and maintenance of the tumor along with MAPK, PI3K and RaGEF (Ral Guanine nucleotide Exchange Factor) signaling pathways [21]. YAP1 (Yes associated protein) is a transcriptional co-activator that participates in several context-dependent transcriptional programs that regulate organ size and promote cell proliferation [22]. It is known to play a role in the development of multiple cancers as a transcriptional regulator of the Hippo Signaling Pathway and maybe serving as a potential target for cancer treatments [23]. The converging of KRAS and YAP1, for the regulation of EMT and tumor survival is investigated in this study. YAP1's ability to prevent anomalous changes in the KRAS mutant cells were proved by the suppression of KRAS expression by YAP1 in CRC cell lines [22]. Due to the fact that YAP1 rescued CRC cell lines from KRAS suppression acting as a substitute, it is proven that YAP1 signaling functionally replaces

KRAS in KRAS-dependent cancer cells [22]. Additional testing was conducted by replacing KRAS with other oncogenes. To clarify the relationship between YAP1 and KRAS even further, it was found that the expression of two YAP1 specific shRNAs abrogated KRAS-driven anchorage independent colony formation [22]. In terms of YAP1's role in the process of EMT, it was shown that both KRAS and YAP1 expression strongly induced the expression of the mesenchymal genes [Vimentin (VIM), Fibronectin (FN1), Slug (SNAI2), and Zinc-finger E-box binding homeobox 1 (ZEB1)] contrary to the reduced expression of epithelial genes [E-cadherin (CDH1) and Occludin (OCLN)] [22]. Hence proving the regulation of EMT by YAP1 and the enrichment of EMT canonical pathway by YAP1 [22].

Cyclin-dependent kinase 8 (CDK8), a protein encoded by cyclin-dependent kinase (CDK) is a colon cancer oncogene, where its' expression was shown to depend on KRAS mutation [24,25]. Study showed lower CDK8 expression in the wild type KRAS pancreatic samples compared to mutated KRAS samples [25]. It has shown that mutated KRAS stimulates CDK8 expression possibly by the up regulation of HIF- α (hypoxia inducible factor) via phosphorylation [25-27]. Both CDK8 and mutated KRAS stimulated the EMT based on the suppression of E-cadherin expression and enhancement of N-cadherin and vimentin expression [25]. Additionally, overexpression of KRAS or CDK8 stimulated cancer cell proliferation, invasion and migration whereas knockdown of either gene yielded opposite results and promoted apoptosis [25]. Prior studies have promulgated the role of CDK8 in the co-activation β -catenin driven transcription in colon cancers characterized by both high CDK8 expression and β -catenin activity [24]. CDK8 promoted the expression of β -catenin by regulating Axin2 and GSK-3 β [24]. In conclusion to this study, mutated KRAS activates CDK8 to stimulate EMT through Wnt/ β -catenin signaling pathway by the upregulation of downstream SNAIL1 (Zinc-finger protein) and ZEB1 genes (Zinc finger E-box binding homeobox protein) that are significant in metastasis, poor survival and pathological angiogenesis [25].

Polycomb Repressive Complex 2 (PRC2), resembles oncogenic or tumor suppressing properties, acting as a barrier to KRAS driven inflammation and EMT in Non-small-cell lung cancer [28]. Lung being one of the primary targets of CRC metastasis, this study can be good lead to understanding EMT in CRC. The study displayed that PRC2 is modulated by either the overexpression of Enhancer zeste homolog 2 (EZH2) or the deletion of Embryonic Ectoderm Development (Eed) enhancing KRAS driven adenoma genesis and inflammation [28]. EZH2 is part of the PRC2 which is responsible for the repression of many genes involved cell differentiation and development [28]. Additionally, the inactivation of Trp53 (p53) activated EMT autonomously in the cell. PRC2 inactivation during KRAS driven tumorigenesis caused tumor acceleration. PRC2 activation on the other hand, induced sterile inflammation in KRAS Non-small-cell lung cancer, phenotypically altering the microenvironment, thereby rewiring tumor progression and severely impairing lung tissue function [28]. Lung tumor cells secrete several cytokines, whose expression levels are changed upon PRC2 inhibition. Granulocyte colony stimulating factor (G-CSF) and Interleukin-6 (IL-6), cytokines upregulation was seen in experiments where PRC2 was inhibited. The cooperation between oncogenic KRAS and transcriptional regulation by PRC2 is uncovered in this study [28].

Novel transcription factors involved in EMT

Signal transducer and activator of transcription 3 (STAT3) is found to be involved in EMT by regulating the transcriptional regulators E-cadherin, the biomarker of EMT [29]. STAT3 Transcription factor is widely known for its partial role in advocating tumor invasion, angiogenesis, tumor cell proliferation and maintaining a pro-carcinogenic inflammatory environment [30]. The active phosphorylated state of STAT3 was also seen increased in hypoxia environment. When HIF-1 α expression is inhibited, hypoxia can no longer induce EMT [29]. The interaction of STAT3 with other transcription factors like TWIST, SLUG and SNAIL that regulates E-cadherin was also explored [29]. The data proved the reduction of EMT with the silencing of STAT3, implying its importance in hypoxia induced EMT. HIF-1 α mRNA levels did not increase in hypoxia, on the contrary it declined significantly with the silencing of STAT3 [29]. Hypoxia of the tumor being one of the fundamental causes that triggers EMT involved in metastasis, STAT3 is important in hypoxia-induced EMT [29]. Although esophageal cells were exploited, the study targeted on the molecular aspects such as the biomarkers of EMT which is why STAT3 has the potential to be examined in further research in the metastasis of CRC [29,30].

Sex determining region Y box 2 (SOX2) comprising in the SRY-related HMG-box factor family is a transcription factor associated with EMT, promoting liver and lymph node metastasis [1]. A study on this transcription factor was coordinated relating to metastasis of CRC. Robust expression of SOX2 is associated with several types of human solid tumors [31]. The metastatic potential of CRC was focused by the inhibition of Physcion an anthraquinone derivative in vitro via the suppression of SOX2 [31]. The expression levels of transcription factor SOX2 in the cells were modulated with shRNA targeting SOX2 and SOX2 overexpressing plasmid [31]. Cell lines treated with physcion projected morphological changes along with the upregulation of E-cadherin mRNA, an epithelial biomarker, compared to decreased level in N-cadherin, vimentin, fibronectin and α -SMA (Alpha smooth muscle actin) which are mesenchymal biomarkers [31]. For a further understanding of the study's relation to metastasis and EMT, the effect of physcion on the regulatory transcriptional repressors (SNAIL, SLUG & TWIST) were inspected. SNAIL & SLUG categorized in Zinc-finger protein family and TWIST1 in bHLH factor family, they are acknowledged in significance to nodal invasion, chemo resistance and poor prognosis [1]. As a result, Physcion modulated the expression of the molecules from observing the mRNA levels [31]. Summing up the results from this study, identified a major role of physcion -induced SOX2 downregulation in the attenuation of the metastatic potential of CRC cells by the activation of ROS/AMPK/GSK3 signaling [31]. Previous studies have exploited the connection between metastatic tumors and ROS (reactive oxygen species) production [31]. Physcion induced ROS generation and stimulated AMPK (5' adenosine monophosphate-activated protein kinase) signaling were found to be inhibited by the metastatic potential in CRC cells [31]. Alternate study implemented prior to the one discussed also demonstrated on how the expression of SOX1 predicts liver and lymph node metastasis in CRC patients [32]. The knockdown of SOX2 induced Mesenchymal-Epithelial Transition. Furthermore, MMP2 activity and Wnt signal pathway activity was significantly decreased along with reduced β -catenin nuclear translocation

[32]. The role of SOX2 in the EMT is mediated through the Wnt Pathway by reducing the expression of E-cadherin at plasma membrane, thus reducing the binding capacity of the adhesion complex for β -catenin [32]. SOX2 was also identified as a novel target of miR-371-5p acting as a switch for EMT [33]. SOX2 can be added to the list of crucial transcription factors and regulators that takes part in tumorigenesis, invasion and migration for CRC.

KLF8 (Kruppel-like factor) is an emerging transcription factor that takes part in invasion and metastasis of colorectal cancer [34]. Studies have identified KLF8 as a regulator of FHL2 (Four-and-half-LIM protein 2), a critical inducer of the EMT and its role towards tumor proliferation [34]. The overexpression of FHL2 domain protein is predicted to be regulated by KLF8 in maintaining the malignancy in CRC cells. KLF8 overexpression promotes EMT in CRC cell lines and induces a shift in the expression of epithelial marker (E-cadherin) to mesenchymal markers (vimentin and N-cadherin) [34]. TGF- β 1 induces mesenchymal marker, vimentin [34]. Blocking TGF- β 1 significantly suppressed endogenous KLF8 expression and TGF- β -induced KLF8 expression in CRC cells. A positive correlation has been reported between KLF8 and FHL2 expression [34]. MET (Mesenchymal epithelial transition) a process which is the reverse of EMT was shown in KLF8 overexpressed cells with FHL2 knockdown. Also notable was the downregulation FHL2 in KLF8 overexpressing cells led to a decreased invasion potential of KLF8-overexpressing cells. [34]. It is identified that FHL2 is a direct transcriptional target of KLF8 due to its direct binding to the FHL2 promoter. Thus transcription factor KLF8 has been identified to play an important role in tumor

progression and a favorable target for the intervention against CRC cell proliferation and metastasis [34]. FHL2 suppression has been investigated for inducing cell differentiation in gastric and colon carcinogenesis [35]. Increased expression of E-cadherin is associated with FHL2 suppression which in turn reduces its interaction with β -catenin [34].

TUSC3 (Tumor suppressor candidate 3), one of the emerging transcription factors that is currently being investigated is known to fall under the category of tumor suppressing genes in context to CRC [36]. Recent studies confirm that TUSC3 promotes CRC progression and epithelial mesenchymal transition through Wnt/ β catenin and MAPK signaling [36]. Embryogenesis and metabolism are the main processes achieved by TUSC3 [36]. TUSC3 expression is found to reversely correlate with NF- κ B (Nuclear Factor) activity [37]. NF- κ B plays a key role in the expression of pro-inflammatory genes including cytokines, chemokines and adhesion molecules [38]. Silenced TUSC3 pancreatic cell lines have exhibited enhanced potential of proliferation, migration and invasion along with liver metastasis in "in vivo" studies [37]. Due to the higher percent of TUSC3 expression present in CRC cells, TUSC3 is proven to be upregulated in CRC [36]. On the contrary, downregulation of TUSC3 significantly suppresses cell growth inhibiting cell proliferation, decreased migratory and invasive abilities [36]. Over expression of TUSC3 causes remarkable decrease in E-cadherin expression and increased vimentin expression [36]. Increased β -catenin and decreased GSK- β (Glycogen Synthase kinase 3) by the over expression of TUSC3 and vice versa has demonstrated through quantitative analysis

Transcription Factor	Role	Correlation to EMT
YAP1	hippo signaling pathway substitute for KRAS	induce mesenchymal markers reduced expression of epithelial markers.
CDK8	Depends upon KRAS mutation Expressed via the up regulation of HIF- α	Activate EMT through Wnt Signaling Pathway by the upregulation of downstream SNAIL, TWIST1 & ZEB1. Suppress E-cadherin Enhance N-cadherin & Vimentin
PRC2	Inhibition of PRC2 caused upregulation of G-CSF & IL-6. Functions as Tumor suppressor. Inactivation led to induction of KRAS driven inflammation.	Negative up regulation of hippo and Wnt signal transduction pathways.
FOXC2	Angiogenesis and Lymph node metastasis. Interact with PIN1, ERK1/2 and PP2A. Transcriptional regulator of PDGFR- β . FOXC2 abundant in G2/M phase. Downregulates CDK1. Causes tumor cells to be resistant to chemotherapeutics via activation of MAPK & AKT pathways.	Promotes ectopic expression of SNAIL, TWIST or TGF- β leading to EMT. Regulation of PDGFR- β that correlates with TGF beta signaling pathway.
FOXM1D	Invasiveness and angiogenesis. Cooperate with ROCK activation. Lymph Node, liver & abdominal metastasis	Regulates expression of VEGF and MMPs. Upregulates regulation of ZEB1/2 and SLUG leading to reduction of epithelial marker E-cadherin.
STAT3	Promotes HIF- α expression. Regulates expression of AKT pathway Upregulated in hypoxic cancer cells.	Hypoxia Induced EMT Interacts with TWIST, SLUG and SNAIL. Decreased epithelial marker and increased mesenchymal markers.
SOX2	Associated with liver and Lymph Node metastasis Promotes sensitivity to chemotherapeutics. Novel target of miR-371-5p Reduced Wnt pathway activity.	Upregulation of epithelial markers and down regulation of mesenchymal markers. Downregulation of SOX2 promoted ROS/AMPK/GSK3 signaling. Knockdown of SOX2 promoted MET.
KLF8	Regulates expression of FHL2 FHL2 regulated by KLF8 through GT Box located on FHL2 promoter.	Over expression of KLF8 shifted epithelial markers to mesenchymal markers. Increased expression of E-cadherin with FHL2 suppression.
TUSC3	Colorectal cancer proliferation & EMT Migratory abilities Downregulation caused significant decrease in phosphorylation of AKT and ERK	Promotes EMT through Wnt & MAPK signaling. Decrease in E-cadherin and increase in Vimentin. Overexpression caused abundance of β -catenin and decreased GSK- β .

Table 1. Key Transcription Factors and the correlation to EMT.

studies [36]. The intracellular location of β -catenin and TUSC3 between their co-expression determined the role of TUSC3 in Wnt/ β -catenin signaling pathway by the direct interaction with the core component of canonical Wnt signaling [36]. Lastly, down regulation of TUSC3 causes significant decrease in the phosphorylation levels of AKT & ERK $\frac{1}{2}$, whereas TUSC3 over expression causes sustained phosphorylation [36]. The validation of the role of TUSC3 in the promotion of proliferation, migration and invasion of CRC cells through the activation of MAPK, PI3K/AKT and Wnt/ β -catenin signaling pathways is confirmed [36]. Further molecular studies regarding the identification of TUSC3 target proteins and the intrinsic mechanism of TUSC3 induced carcinogenesis should be provoked to understand the metastatic process in CRC [36].

Discussion

Transcription factors are the vital components involved in cell metabolism, proliferation and programmed apoptosis. Emerging studies scrutinizing the transcription factors and their roles in the metastatic processes in CRC provides a new insight on tumor invasion and proliferation. The target proteins encoded by oncogenic transcription factors (Table 1) and the associated co-regulators shows a wide array of opportunities in the future of adjuvant therapies. EMT and MET markers such as E-cadherin, N-cadherin and vimentin are targeted to explore its' correlated transcription factors attributing to the metastasis of cancer. EGFs and VEGFs initiates signal cascades triggering the metastasis related transcription factors initiating the transcription of proteins and protein receptors leading to angiogenesis and cell dedifferentiation. Signal transduction pathways are constantly activated and inhibited by transcription factors aiming for cell proliferation or apoptosis. The silencing or over expression of transcription factors thus play a crucial role in the suppression or over growth of tumors as has been documented in many in vivo and in vitro study models.

Therefore, it is crucial to know the pathways and their specificity towards tumor cell suppression or proliferation. As there are many transduction pathways being either one-way or reversible, in depth analysis of the target proteins synthesized by the activation of these pathways primarily from the transcription factors is vital to prevent metastasis. Majority of research should continue to focus on the molecular mechanisms that plays a crucial role to accelerate the cell's adaptive ability towards dedifferentiate which is the first step for the initiation of epithelial mesenchymal transition.

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