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Microbes-induced EMT at the crossroad of inflammation and cancer

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It is noteworthy that bacterial or viral infections, and the resulting chronic inflammation, have been shown to predispose individuals to certain types of cancer. Remarkably, these microbes upregulated some transcription factors involved in the regulation of the epithelial to mesenchymal transition, referred herein as EMT. EMT is a cellular process that consists in the conversion of epithelial cell phenotype to a mesenchymal phenotype. Under physiological conditions EMT is clearly important for embryogenesis, organ development, wound repair and tissue remodeling. However, EMT may also be activated under pathologic conditions, more particularly in carcinogenesis and metastatic progression. In this review, we make a parallel between microbes- and growth factors-induced transcription factors. A unifying EMT model then emerges that may help in understanding the development of microbial pathogenesis and in defining new potential future therapeutic strategy in treating diseases linked to infections.

Introduction

Epithelia are physical barriers that constitute a functional interface between distinct body compartments and the outside. Under healthy condition, cells that composed the epithelial sheets are tightly bound to neighboring cells and to underlying basement membranes via various structures such as adherens junctions, tight junctions, desmosomes and hemi-desmosomes.[1] However, epithelial cells empower high degree of plasticity and under certain circumstances such as developmental processes, fibrogenesis or tumor progression, they lose their static phenotype and acquire migratory and invasive behavior.[2] Epithelial plasticity could be limited to relocalization of junctional proteins or to a more drastic epithelial to mesenchymal transition (EMT). EMT is associated with phenotypic and genotypic changes. Phenotypically, epithelial cells undergoing an EMT lose their cobblestone phenotype to acquire an elongated fibroblastic morphology. Genetically, a downregulation of E-cadherin, as well as downregulation and translocation of β-catenin from the cell membrane to nucleus and an upregulation of mesenchymal markers such as vimentin, fibronectin and N-cadherin were observed. Overall, these changes allowed a disassembly of cell-cell junction, actin cytoskeleton reorganization and induction of contractile proteins and non-motile epithelial cells convert into individual, motile and invasive mesenchymal phenotypic cells.[3-5]

It is to note that EMT is different than collective cell movement, which occurs when two or more cells that retain their genetic and phenotypic feature move together across a two-dimensional (layer of extracellular matrix) or through a three-dimensional interstitial tissue.[6]

During the last decade major efforts have been made to decipher molecular signals that control initiation of EMT. It appears that EMT is the result of growth factor-induced signaling pathways that affect the epithelial integrity and target downstream transcriptional regulators to regulate epithelial to mesenchymal gene expression. Mostly, these signaling pathways share common endpoints, the central target being the regulation of expression of the adherens junction protein, E-cadherin. Remarkably some of these EMT-signaling pathways are upregulated by microbial pathogens, therefore suggesting that pathogens may also be considered as EMT inducers. In addition, the observation that microbe invasion leads to transforming growth factorβ (TGFβ) modulation supports our proposal.[7-9] Indeed, once activated, the TGFβ receptor leads to phosphorylation and activation of two transcription factors, Smad-2 and Smad-3.[10] Phospho-Smad2/3 heterodimerize with Smad-4 and the Smad-complex translocate to the nucleus to regulate the transcription of genes that control cell proliferation, differentiation and cell migration.[11] Moreover, TGFβ activates Smad-independent signaling cascade leading to the activation of the classical Ras-MAPK pathway,[12] a signaling pathway that is particularly relevant for the EMT process.

The Epithelial to Mesenchymal Transition

A general overview. EMT has been extensively reviewed in the literature,[3-5] we therefore decided to summarize the key point steps of this cellular process in **Figure 1**. As mentioned earlier, epithelial cells are apico-basal polarized cells with lateral adherence to their neighbors under the control of E-cadherins and basal adherence to the extracellular matrix (ECM) mainly under the control of cytokeratins. In contrast, migrating mesenchymal cells display front-back polarity with only focal adhesions to their neighbors and to ECM. These contacts are mainly under the control of vimentin. Therefore, loss of E-cadherin and cytokeratin expression and gain of vimentin are commonly used to characterize EMT.

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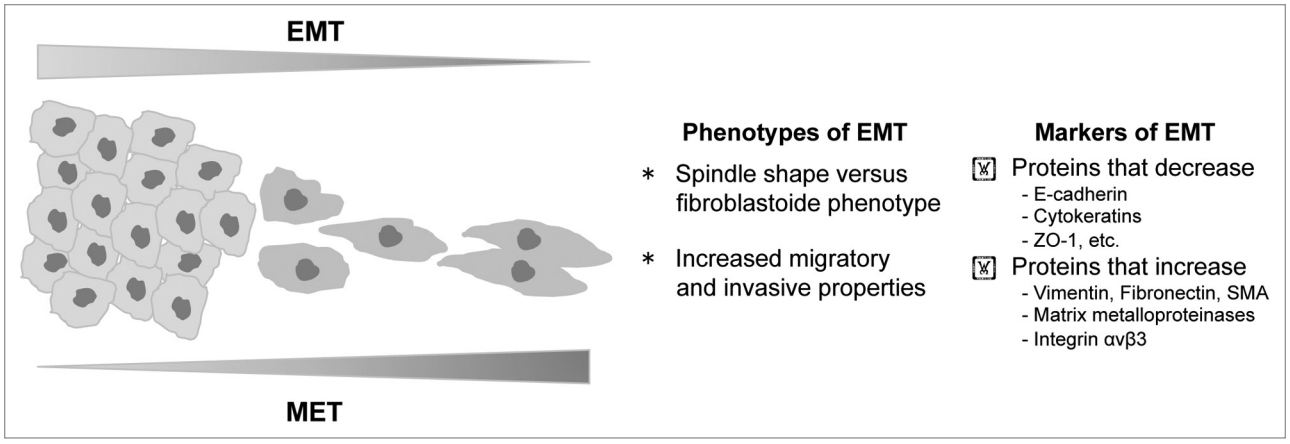

Figure 1. A basic view of epithelial to mesenchymal transition. EMT is a highly conserved and fundamental process that governs morphogenesis in multicellular organisms. EMT is the result of a transcriptional repression of E-cadherin gene leading to the loss of the epithelial phenotype and the remodeling of the actin cytoskeleton associated to the mesenchymal phenotype. EMT plays an important role in the emergence and progression of carcinoma by allowing carcinoma cells scattering. This cellular process is reversible; at secondary sites, solitary carcinoma cells can extravasate and form a new carcinoma trough a mesenchymal to epithelial transition (MET).

Transcription factors and EMT. EMT is controlled by a small group of transcription factors defined as the core EMT regulatory factors that comprises SNAI1/Snail1,[13] SNAI2/Slug/Snail2,[14] ZEB1, Sip1/ZEB2 [15] and Twist1 and 2.[16] Whereas these proteins share the same function that is a transcriptional repression of E-cadherin, they have different structures (**Fig. 2**). The Snail family is composed of zinc finger proteins, the ZEB family has two zinc finger clusters and Twist proteins have a helix loop helix motif.[17] Interestingly enough, it was recently shown that in neural crest cells all these factors are coordinately regulated by an E3 ubiquitin ligase named Partner of paired (Ppa).[18] Ppa is a F-box containing protein that targets its bound substrates to the ubiquitin-proteasome system for degradation. Given the importance of EMT in physiological development, the existence of a common regulatory protein that can be tightly controlled in a spatio-temporal manner makes sense. However, it remains to be defined whether Ppa is also involved in pathophysiogical EMT such as tumor progression and microbial pathogenesis.

In addition to the classical core EMT regulatory factors, the Foxo3a protein, which belongs to the Forkhead transcription factor family,[19] is known to upregulate E-cadherin expression via downregulation of Twist 1.[20] Further, the lipopolysaccharide (LPS), a major component of the outer membrane of Gram-negative bacteria, has been shown to regulate the Foxo3 protein activity in intestinal epithelial cells,[21] suggesting a direct link between LPS and non-classical regulation of E-cadherin expression.

The signaling pathways that govern EMT. Growth factor binding to their respective receptors triggers activation of a multitude of signaling pathways that ultimately stimulate the core EMT regulatory factors.[12] Similarly, microbes via their specific trans-membrane receptors induce cell signaling that mediate transcription factor activation. Interestingly, growth factors and microbes share common signaling pathways, suggesting that microbes may be considered as EMT inducers.

The IKK complex. The IKK complex (700 to 900 kDa) consists of two catalytically active kinases (IKKα and IKKβ) and a regulatory scaffold protein, IKKγ (NEMO), which connects both of the catalytic subunits with upstream activators. In non-stimulated cells, NFκB complexes are sequestered in the cytosol in an inactive form, due to their association with inhibitory IKB proteins (IKBα, IKBβ and IKBε). Upon activation, activated IKK kinases promote the degradation of IKBα and NFκB family of transcription factors can than translocate to the nucleus where they regulate gene expression.[22] The NFκB family of transcription factors which is composed of five members, p65 (REL-A), REL-B, cytoplasmic (c) REL, p50;p105 (NFκB1) and p52;p100 (NFκB2), is widely activated under cytokines and/or microbial challenge.[23,24]

In an integrative genomic analysis, NFκB has been shown to regulate ZEB2, a regulator of EMT.[25] In addition, NFκB is involved in the upregulation of *twist-1* and *twist-2* expression in response to TNFα; this regulation is lost in fibroblasts lacking the p65 subunit of NFκB.[26] Moreover, the authors proposed a model in which TWIST orchestrates a negative feedback loop by repressing cytokine expression under cytokine challenge and therefore maintaining a controlled inflammatory response. Interestingly enough, the classical NFκB pathway is also responsible for the EMT process attributable to von Hippel-Lindau (VHL) loss and subsequent HIF-1 activation since molecular and pharmacological approaches to inhibit NFκB promote a partial reversion to an epithelial phenotype.[27] Finally, NFκB also controls mesenchymal marker expression since an NFκB binding site has been described on the vimentin gene[28] and overexpression of a constitutively active form of p65 in breast cancer cells increases expression of vimentin.[29] Moreover, NFκB directly activates the transcription of the (MMP)-9 matrix metalloprotease gene, a type IV collagenase which increases cellular invasiveness and motility[30] and indirectly controls MMP-2.[31]

The MAPK module. MAPK signaling pathways are organized in modular cascades in which activation of upstream kinases by cell surface receptors leads to sequential activation of a MAPK

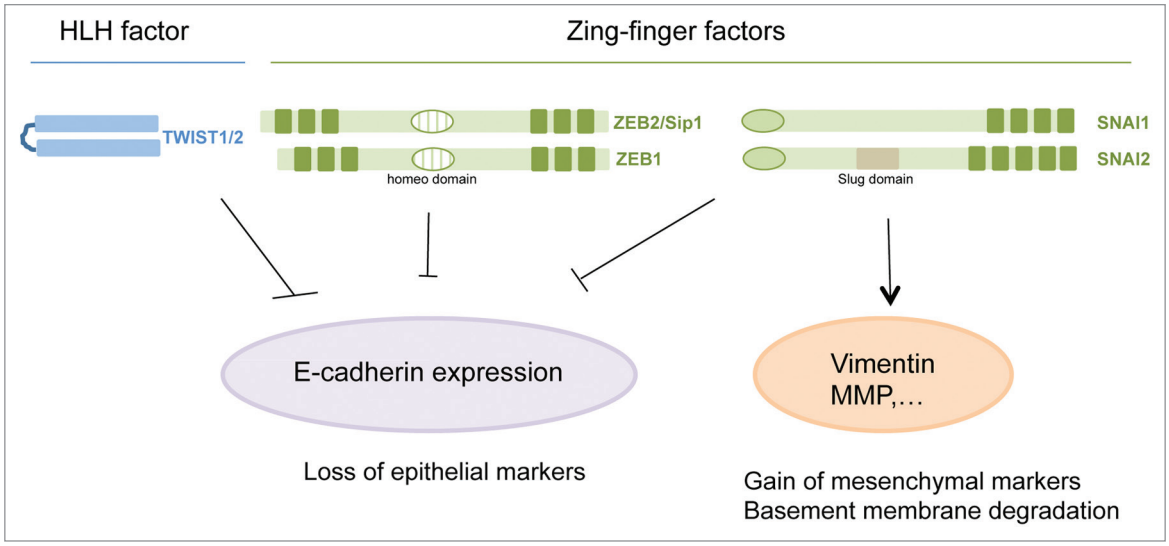

Figure 2. The transcription factors that govern EMT. The core EMT regulatory factors are composed of Helix Loop Helix (blue) and zing-finger DNA binding proteins. The green boxes represent zing-finger clusters. Homeo domains of the ZEB transcription factor family is represented as hatched circle and the Slug domain of SNAI2/Slug1 is represented as a gray motif. Once in the nucleus these transcription factors downregulate E-cadherin expression. The SNAI family of transcription factors can also activate vimentin and fibronectin expression and control the membrane basement degradation.

module (MAPKKK→MAPKK→MAPK). This module comprises three different signaling pathways (MAPK, p38 and JNK). They are activated by inflammation, stress, oxidative stress and mitogens.[32] Ultimately, these interconnected signaling pathways activate transcription factors leading to repression of E-cadherin but also activation of mesenchymal genes and cell motility.[2,33]

The Ras > Raf > MAPK kinase cascade is activated by a large number of mitogen receptors including tyrosine kinase receptors (such as fibroblast growth factor receptor, epithelial growth factor receptor, hepatocyte growth factor, vascular endothelial growth factor) and the G-protein coupled receptors, a family of seven trans-membrane domains proteins including cytokine and chemokine receptors. This signaling cascade, which is extremely well conserved from yeast to man, allows the repression of E-cadherin expression via activation of Snail/Slug. This pathway also controls upregulation of mesenchymal genes and cell motility via activation of SRE, AP1 and SP transcription factors[2] and references herein.

The p38 MAPK pathway is certainly the most relevant MAPK module in regard to microbe sensing. It was first described to downregulate E-cadherin expression during mouse gastrulation.[34] Further, p38 MAPK was described to participate in TNFα-[35] and TGFβ-induced EMT.[36] In addition a crosstalk between the Smad and NFκB pathways accentuates TGFβ-induced EMT in presence of TNFα.

The c-Jun N-terminal kinase (JNK) pathway is mainly activated by cellular stress and by cytokines that act through several upstream kinases such as TAK1 and TRAF6. JNK pathway mediates TGFβ-induced EMT in keratinocytes.[37] Further it was shown that activation of Smad3 by JNK is necessary to mediate TGFβ-induced EMT.[38]

The PI3K/Akt pathway. The oncogenic serine/threonine kinase AKT (also known as PKB), which is a downstream effector of the phosphatidylinositol-3-kinase (PI3K), has been shown to repress transcription of the E-cadherin gene.[39] Indeed, cells producing a constitutively active form of Akt produced Snail, which in turn repressed expression of the E-cadherin gene. In addition, activated Akt triggered loss of cell-cell adhesion, morphological changes, loss of apicobasolateral cell polarization, induction of cell motility and decreased in cell-matrix adhesion, all features that represents the hallmark of EMT. Further, a link with TGFβ signaling via autocrine or paracrine stimulation has been proposed since TGFβ-induced cuboidal morphology to a spindle-like elongated shape was inhibited by the the PI3K inhibitor LY294002 and by a dominant-negative (kinase-inactive) AKT mutant.[40]

The others pathways. The readers should keep in mind that in addition to these signaling pathways others less classical pathways exist. Among them, the smad pathway that is activated by TGFβ[7-9,41] and the signal transducers and activators of transcription (STAT) pathways which are activated by tyrosine phosphorylation of receptor tyrosine kinases, by the cytokine and chemokine receptor/Janus activated kinase (JAK) complexes or by non-receptor tyrosine kinases.[42] In particular, STAT3 has been involved in EMT.[43,44] Finally, an alteration in the micro-environmental oxygen tension (hypoxia) and activation of hypoxic signaling through hypoxia-inducible factor (HIF) [45-51] and microRNAs (miRs) [52-55] are emerging as important triggers and modulators of EMT.

EMT and Bacterial Pathogens

Prior to induce signaling pathways relevant for EMT, microbes should be sensed by the cells. These sensors belong to the pattern recognition receptor families.

A general overview on pattern recognition receptor (PRR). Twenty years ago, Charles Janeway identifies a mechanism based on the recognition of pathogen-associated molecular patterns (PAMPs) by host pathogen-recognition receptors (PRRs). He was the first to understand that this mechanism represents the first defense against pathogens.[56] His discovery was further confirmed by the identification of the Drosophila transmembrane receptor Toll as a key player in the antifungal defense.[57] One year later the human homolog was discovered,[58] and then the toll-like receptor (TLR)4 was identified as the protein involved in the recognition of LPS. Therefore, the link between a microbial motif, LPS and a host receptor, TLR4, was made.[59] Today, TLRs family encounters 10 members in human and each TLR has a distinct function in terms of PAMP recognition.[60]

TLRs are divided into two subgroups based on their cellular localization and respective PAMP ligands. The first group is expressed on cell surfaces and recognizes mainly microbial membrane components such as lipids, lipoproteins and proteins. This group is composed of TLR1, TLR2, TLR4, TLR5, TLR6 and TLR1. The second group, expressed exclusively in intracellular vesicles where the receptors recognize microbial nucleic acids, is composed of TLR3, TLR7, TLR8 and TLR9.

In mammals, in addition to TLRs, an intra-cytoplasmic sensing system for microbial effector exists. This second family of receptors is named Nod (nucleotide-binding oligomerization domain)-like receptors (NLRs). Among the NLRs, Nod1 and Nod2 recognize the intracellular degradation products of bacterial cell wall components such as muropeptides.[61] In this review we will focus our attention on TLRs and NLRs that represent the most important microbial sensors. The readers should keep in mind that other sensors exist.[62-65]

Mediators linking PRRs to transcription factors. TLRs shared a common structure based on three different domains: (1) a type I trans-membrane proteins with extracellular domains containing leucine-rich repeats and mediating the recognition of PAMPs, (2) a trans-membrane domains and (3) an intracellular Toll-interleukin 1 (IL-1) receptor (TIR) domains which recruit TIR domain-containing adaptor molecules to induce downstream signal transduction.

MyD88 was identified as the first member of the TIR family adaptors. Once bound to TLRs, MyD88 recruits the IL-1 receptor-associated kinases IRAK4, IRAK1, IRAK2 and IRAK-M. Mostly, direct or indirect activation of IRAK allows the activation of IKK and MAPK signaling pathways which in turn induce a myriad of transcription factors.[60] The TIR family also comprise TIRAP (Mal), TRAM and TRIF. TIRAP and TRAM function as additional sorting adaptors allowing the recruitment of MyD88 to TLR2 and TLR4. TRIF is also used by TLR3 and TLR4 and induces alternative pathways that lead to activation of the transcription factors IRF3 and NFκB.

RIP2 (also known as RICK or CARDIAK) is the common downstream signaling molecule of Nod1 and Nod 2.[66] RIP2 leads to the activation of NFκB via its binding to NEMO, the regulatory subunit of the IKK complex.[67] We schematized in **Figure 3** the PRR-induced signaling pathways. Thus, activation of IKK and MAPK and PI3K/Akt signaling pathways appeared to be the common end points of microbes sensing. However, cellular responses to invading microbes are also the result of indirect activation of intracellular pathways comprising Smad and Stat pathways but also hypoxia and microRNAs.

The microbes normally present in humans are collectively estimated to number 10-fold that of human cells. Mainly located in the gut, the microbiota is crucial for human life by influencing human physiology and nutriment uptake.[69] In addition, the microbiota contributes to the shaping of healthy intestinal immune responses.[70] It has been proposed that an alteration in the development and/or composition of the microbiota may disturb the relationship between microbes and the immune system. In turn, immune defects may favor pathogenesis of various human inflammatory disorders,[71] and inflammatory disorders promote EMT. We can therefore speculate that most of microbes that persist in the body have the potential to indirectly favor an EMT behavior. In this review we will only focus on the few examples that describe a direct involvement of microbial pathogens in EMT induction based on their ability to modulate E-cadherin expression via an increase of the core EMT regulatory factors. Indeed, we will let aside all bacteria species and viruses that only induce a loss of epithelial barrier functions.

Bacterial products. *LPS.* Lipopolysaccharide (LPS), the major component of the outer membrane of Gram-negative bacteria binds to TLR4. LPS is an endotoxin, which induces a strong response from normal animal immune systems; therefore it is widely used to study gram-negative bacteria-induced cellular responses. Intriguingly, we found in the literature only one report that studies LPS-induced EMT. Using a model of intrahepatic biliary epithelial cells, Zhao and co-authors have shown that in response to LPS stimulation a decrease in E-cadherin expression was observed whereas expression of the mesenchymal markers (S100A and α-SMA) increased by more than 12-fold.[72] In addition to EMT markers, they noticed that the messenger coding for TGFβ-1 was significantly increased. As indicated previously, TGFβ-1 is a well-known inductor of EMT that transmits its effect via Smad2/3. Indeed, silencing of Smad 2/3 in biliary epithelial cells resulted in a significant decrease of mesenchymal markers and an increase in E-cadherin expression. Therefore, the authors concluded that LPS induced the EMT probably through the TGFβ1/Smad2/3 pathway. In addition and as described in an earlier section, LPS also induced phosphorylation of the Foxo3 protein leading to its export from the nucleus and its degradation in the cytosol.[21]

Flagellin and muramyl dipeptides. Flagellin and muramyl dipeptides (MDP) represent the two main other bacterial products that have been extensively studied in the literature. Once bound to the TLR5 receptor, flagellin induced the NFκB and MAPK signaling pathways.[73] As described for LPS, cells induced by bacterial flagellin produced TGF-β1 and TGFβ is an EMT inducer.[74] However, it remains to be determined whether flagellin are true EMT inducers. Similarly, once in the cells, MDP are recognized by the NOD proteins which in turn induced NFκB and MAPK signaling.[75] A recent report claims that MDP induce the expression of genes associated with EMT and invasive cell growth in intestinal epithelial cells (Scharl M., oral presentation

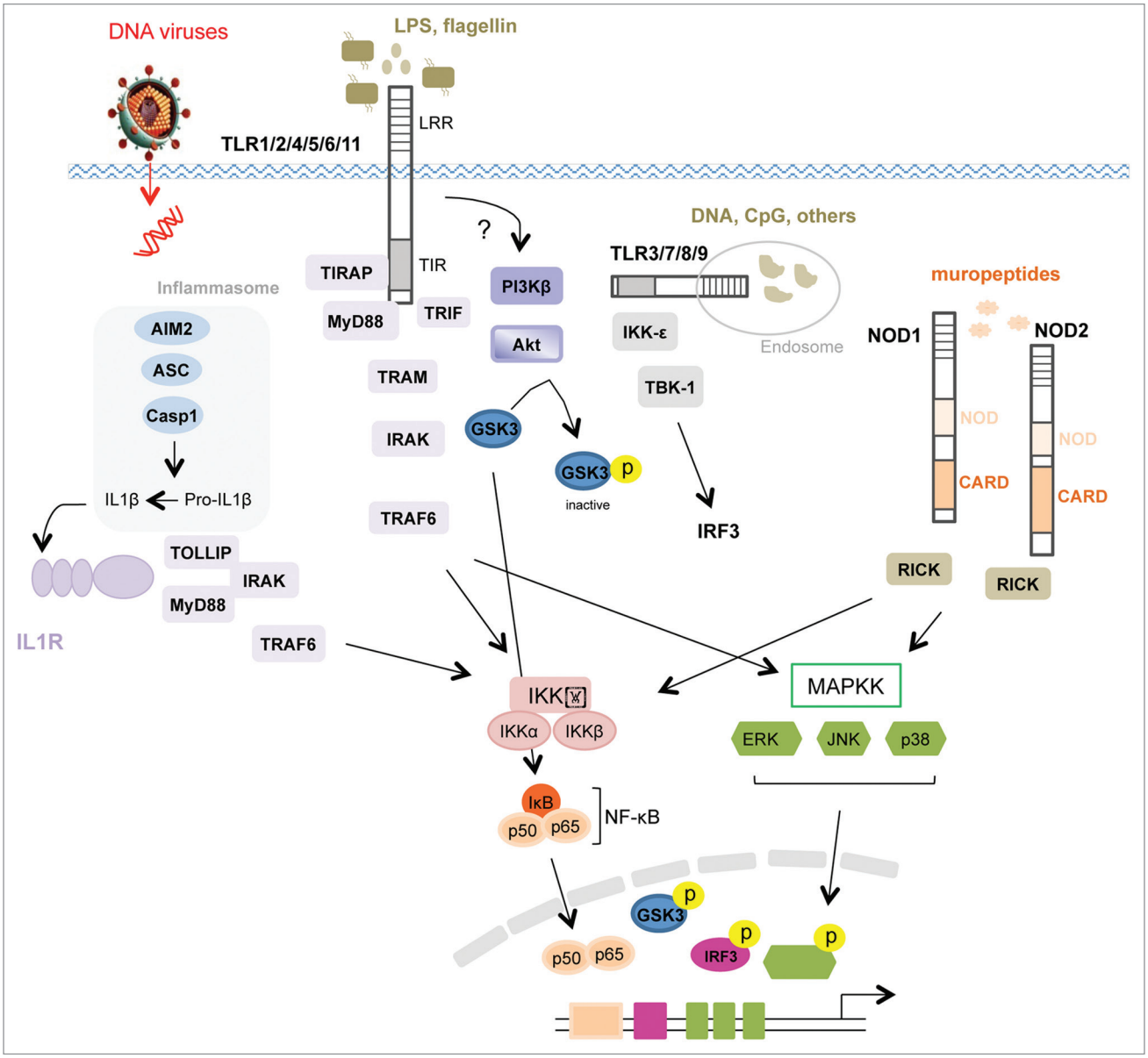

Figure 3. A schematic view of PPR-induced pathways involved in stimulation of NFκB and MAPK signaling. TLRs (TLR1, TLR2, TLR4, TLR5, TLR6, TLR7 and TLR9) do activate IKK and MAPK modules by binding of MyD88 to the receptor TIR domain and subsequently triggering IRAK, TRAF6 and TAK1. Alternatively, TIRAP (TIR domain-containing adaptor protein), a second TIR-domain-containing adaptor protein, is involved in the MyD88-dependent signaling pathway through TLR2 and TLR4. A third TIR-domain-containing adaptor, TRIF (TIR domain-containing adaptor protein inducing IFNβ), is essential for the MyD88-independent pathway. Further, the non-typical IKKs IKKε and TBK1 (TRAF-family-member-associated NFκB activator (TANK)-binding kinase 1) mediate activation of IRF3 downstream of TRIF. A fourth TIR-domain containing adaptor, TRAM (TRIF-related adaptor molecule), is specific to the TLR4-mediated, MyD88-independent/TRIF-dependent pathway. By contrast, activation of NLRs leads to the recruitment of the receptor-interacting protein 2 (RIP2) kinase, which is essential for the activation of the IKK complex. In addition, activation of NOD1 leads to JNK stimulation. Finally, double strand DNA has been linked to inflammasome activation. This protein complex, which is composed of NLRs of the NALP-family and adaptor-proteins apoptosis-associated speck-like protein (ASC), mediates the generation of IL-1β through cleavage of its precursor by caspase-1. Upon IKK complex activation, NFκB is freed and consequently translocate to the nucleus where it can bind to the promoter of its targeted genes. Similarly, once activated, ERK, JNK and p38 kinase translocate to the nucleus where they phosphorylate their respective transcription factors and therefore modulated gene expression. TLRs are connected to the PI3K/Akt pathway. Indeed, depending of the TLRs and the cells, PI3K has been shown to modulate transcription factor activities and cytokine production.[68] Up to date, the molecular link between TLRs and PI3K are still unknown.

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Helicobacter pylori. *Helicobacter pylori* is a gram-negative bacteria which colonizes the human stomach of about 50% of the world's population. Highly purified lipopolysaccharide from *H. pylori* strain 26,695 activate NFκB in HEK293 via TLR2 but not TLR4. Further in gastric epithelial cells, *H. pylori* induce TLR2 and TLR5 signaling pathways leading to NFκB

activation.[76] In addition to TLRs, *H. pylori* signal to cells via their numerous virulence factors. Two of them, the cytotoxin VacA and CagA, an effector of the cag pathogenicity island, can co-opt epithelial cell function. Whereas VacA can disrupt the barrier function of tight junction,[77] CagA has major effects on the apical junctional complex allowing the deregulation of epithelial cell-cell adhesion and a loss in epithelial polarity.[78,79] More importantly, using the pathogenic *H. pylori* strain 60190, Yin and co-authors observed expression of Snail and Slug in gastric epithelial cells.[80] Further, they demonstrated that induction of EMT genes depends on *H. pylori*-induced signaling cascade pathways that involve gastrin, MMP7 and shedding of soluble heparin-binding epidermal growth factor. Interestingly, the increase of gastrin observed in response to *H. pylori* infection occurred via a Ras > Raf > Mek > Erk > NFκB signaling pathway.[81] Then, it appears that NFκB is a central common effector that plays a key role in the EMT process induced by pathogenic *H. pylori*.

Enterovirulent *Escherichia coli* strains. *Escherichia coli,* which colonize the gastrointestinal tract of human infants within a few hours after birth, normally coexist in harmony with its human hosts. However, there are several highly adapted *E. coli* clones that have acquired specific virulence factors, which confer an increased ability to adapt to new niches and allow them to cause a broad spectrum of diseases. Among the intestinal pathogens there are six well-described classes: enteropathogenic-, enterohemorrhagic-, enterotoxigenic-, enteroaggregative-, enteroinvasive- and diffusely adherent-*E. coli*. Enteropathogenic *E. coli* cause entero/diarrheal disease as a consequence of lack of intestinal barrier permeability.[82] In most of the cases this epithelial plasticity is limited to relocalization of junctional proteins; however, depending on the bacterial strain used to infect epithelial cells, it could lead to a more drastic EMT. Among the families of entero-pathogenic *E. coli*, diffusely adherent *E. coli* (DAEC) is a heterogeneous group with variable virulence factors promoting adherence to epithelial cells.[83] Using the clinical isolate DAEC C1845, we have shown that infection of intestinal epithelial cells promotes an EMT-like behavior. We have deciphered the molecular mechanisms leading to EMT and observed that F1845 adhesin binding to the DAF receptor promotes Ras > Raf > MAPK and PI3K pathways.[84-86] Activation of these signaling pathways is required to induce an increase in HIF-1α protein expression but also Twist1 mRNA expression. We noticed that HIF-1α silencing significantly blocked the expression of Twist1 gene, revealing a role for HIF-1 in the transcriptional regulation of this gene. Furthermore, we observed that C1845-induced HIF-1α protein expression leads to a loss of E-cadherin and cytokeratin 18 and an increase in fibronectin expression, which are reversed in HIF-1α silenced cells,[48] therefore highlighting the critical role of HIF in DAEC-induced EMT.

EMT and Viral Pathogens

As for microbial pathogens, viral infection leads to activation of intracellular signaling pathways;[87] thus we can intuitively speculate that viruses can induce EMT. The major pathogenic viruses include cytomegalovirus (CMV), herpes simplex virus (HSV), Epstein-Barr virus, Kaposi sarcoma-associated herpes virus, polyoma virus, hepatitis B and C virus and human papilloma virus. Previous works indeed confirmed that at least two families of viruses (Epstein-barr and hepatitis B and C) induce EMT in epithelial cells.

Epstein-barr virus. Epstein-Barr virus (EBV) is a member of the herpes virus family, which infects more than 90% of world population. EBV utilizes normal B cell biology to infect, persist and replicate in B cells. In these cells, EBV initially uses TLR7 to enhance cell proliferation.[88] Further, it was shown that EBV infection of primary human monocytes induced the release of monocyte chemotactic protein 1, a cellular response mediated by TLR2.[89] Beyond immune cells, EBV also infects epithelial cells and it has been associated with neoplastic diseases such as nasopharyngeal carcinoma;[90] the link between EBV and EMT has been studied in this particular context. Latent EBV encodes for eight proteins, two of them, the latent membrane protein 1 and 2A (LMPs), which highjack cell host signaling,[91,92] are particularly involved in EMT. Horikawa and coauthors were the first to describe that transformation of MDCK epithelial cells with LMP1 induces EMT, characterized by loss of epithelial markers, gain of mesenchymal markers and its associated increase in cell motility and invasiveness.[93] To go further, the authors have shown that Twist1-silencing in MDCK cells resulted in changes from scattered and fibroblast-like shapes to tightly packed cobblestone morphology, characteristics of mesenchymal-to-epithelial transition, the reverse of EMT. Finally, the authors demonstrated that LMP1 induces Twist through NFκB in nasopharyngeal epithelial cells. More recently the same group demonstrated that Snail1 acts in combination to twist1 to induce EMT.[94] Similar results were found in alveolar epithelial cells.[95] In addition, a link between EBV and fibrosis was demonstrated with EMT being the core of the process. Indeed, LMP1 induces pro-EMT signaling that occurs primarily through the nuclear factorκB pathway and secondarily through the extracellular signal-regulated kinase (ERK) pathway.[96]

In addition to a classical effect on intracellular signaling, EBV also downregulates expression of miR-200a and miR-200b, the downregulation of which induces EMT.[97] First, the authors demonstrated an association between miR-200a and miR-200b downregulation and E-cadherin expression on resected gastric carcinoma tissue. Further, using in vitro established EBV-infected cell lines they confirmed that downregulation of these miRs correlates with upregulation of the ZEB family of transcription factors and their associated loss of cell-to-cell adhesion. Finally they uncovered the ability of LMP2A, EBNA1 and BARF0 to downregulate the pri-miR-200 transcript.

Hepatitis B and C viruses. At least seven different viruses cause hepatitis, hepatitis viruses A, B and C are the most known. Whereas hepatitis virus A (HAV) induces acute infection disease of the liver, HBV and HCV induce more chronic diseases that can lead to cirrhosis and hepatocellular carcinoma. Both HBV and HCV have been shown to induce EMT.

Viral particles of mammalian HBV encode for a small regulatory protein, known as the X protein that modulates intracellular signaling pathways by directly or indirectly interacting with host factors. Therefore it was hypothesized that HBV X protein may

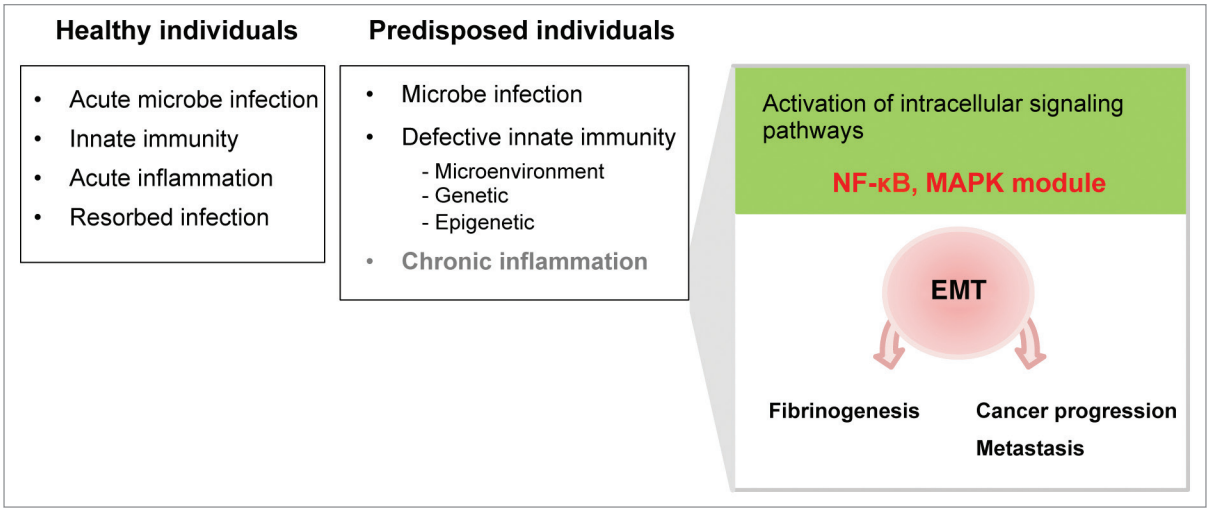

Figure 4. Microbe-induced chronic inflammation in predisposed individuals leads to EMT. Here we suggest a model in which microbe infection plays a critical role as an EMT promoter. In healthy individuals, microbe infection is contained by the innate immunity. By contrast in predisposed individuals the innate immunity is exceeded by microbe infection leading to chronic inflammation. Chronic inflammation, associated to chronic infection lead to sustained NFκB and MAPK module activation: the basement of EMT. Finally, EMT plays a critical role in onset of various human pathologies such as fibrinogenesis, cancer progression and metastasis.

induce EMT in hepatocytes. To test this hypothesis Yang and coauthors transfected hepatocytes with HBx gene and observed that cells underwent morphological changes from an epithelial morphology to spindle-like shape associated with an increase in invasive potential.[98] When the authors treated the cells with PP2, a well-known inhibitor of the Src kinase family, they noticed that cells recovered their original epithelial morphology. Therefore, they claimed that activated c-Src played a critical role in the HBx-induced EMT of hepatocytes.

HCV core protein, which interacts with various cellular proteins induces host cells responses.[99-101] Of particular interest, HCV core protein interacts with Smad3 and consequently inhibits TGFβ induced Smad3 transcriptional activity.[102] Since the TGFβ/Smad3 pathway induces EMT, it was suspected that HCV core protein directly impacts on the EMT process. Using stably transfected cell lines and primary mouse hepatocytes as well as primary human hepatocytes infected in vitro with lentiviruses encoding HCV core protein, Battaglia and coauthors demonstrated that core protein expression was sufficient to provoke EMT in primary hepatocytes. This effect was reverted by addition of a specific inhibitor of TGFβ I receptor thus demonstrating a TGFβ dependent effect of core on EMT development.[103]

HCV core protein has also been involved in the pathogenesis of cholangiocarcinoma. In agreement with this idea, HCV core protein expression in cholangiocarcinoma cells induces EMT through a mechanism dependent on LOXL2 pathway.[104]

Perspective: EMT and Microbial Pathogenesis

The field of research encompassing EMT has been one of the most exciting areas in embryogenesis, organ development, wound repair and tissue remodeling over the past 10 years. This overview is by no means intended to provide a global view on EMT. Instead, we have attempted to depict the main lines that govern EMT in order to highlight similarities that exist between growth factor-and pathogens-induced signaling pathways allowing us to give a coherent picture of the place of microbial infection in EMT and subsequent human pathologies (**Fig.** 4). However, it is important to note that in healthy individuals, infection is effectively controlled, and the inflammatory response is promptly resolved. Indeed, microbes-induced chronic inflammation is intimately linked to defective innate immunity correlating with microenvironment, genetic and epigenetic susceptibilities but also treatment access. For example, *H. pylori* colonize the human stomach of about 50% of the world's population, however less than 2% of this population will develop a stomack cancer, implying the existence of individual predispositon.

Interestingly, it appears that only pathogens associated to chronic pathologies (fibrinogenesis and cancer)[105] have been described to induce EMT. Given that all pathogen recognition receptors induce IKK and MAPK pathways, one can speculate that each pathogen may have the potential to induce EMT and EMT-linked pathologies, such as cancer, as its attack remains unresolved by innate immunity. Keeping that in mind, we can assume that a large part of EMT knowledge can be moved to translational research in molecular medicine with potential future new therapeutics in treating diseases linked to infections.

Acknowledgments

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