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Epithelial to Mesenchymal Transition in Microbial Pathogenesis

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1. 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 by adherens junctions, tight junctions, desmosomes and hemi-desmosomes (Farquhar, M. G. & Palade, G. E., 1963). However, epithelial cells empower high degree of plasticity and under certain circumstances such as developmental processes, fibrogenesis or tumor progression, they loss their static phenotype and acquire migratory and invasive behavior (Grunert, S., et al., 2003). Epithelial plasticity could be limited to relocalization of junctional proteins or to a more drastic epithelial to mesenchymal transition (EMT) which is characterized by disruption of intercellular contacts, loss of epithelium-specific proteins, switch to a mesenchymal gene expression pattern, and gain of invasive properties (Thiery, J. P., 2002). 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 (Ilina, O. & Friedl, P., 2009).

2. Deciphering the EMT process

2.1 General concept

EMT has been extensively reviewed in the litterature (Nieto, M. A., 2011, Thiery, J. P., 2002, 2009) and we summarized in **Figure 1** the key point steps of this cellular process. As stated above, epithelial cells are apico-basal polarized cells with lateral adherence to their neighbors under the control of E-cadherins. The adhesion sites to extracellular matrix (ECM) are focused to the basal lamina, and cytokeratins are the main intermediate filaments. In contrast,

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migrating mesenchymal cells display front-back polarity with only focal adhesions to their neighbors and to ECM, and have vimentin as a major intermediate filament. Therefore, loss of E-cadherin and cytokeratin and gain of vimentin are commonly used to characterize EMT.

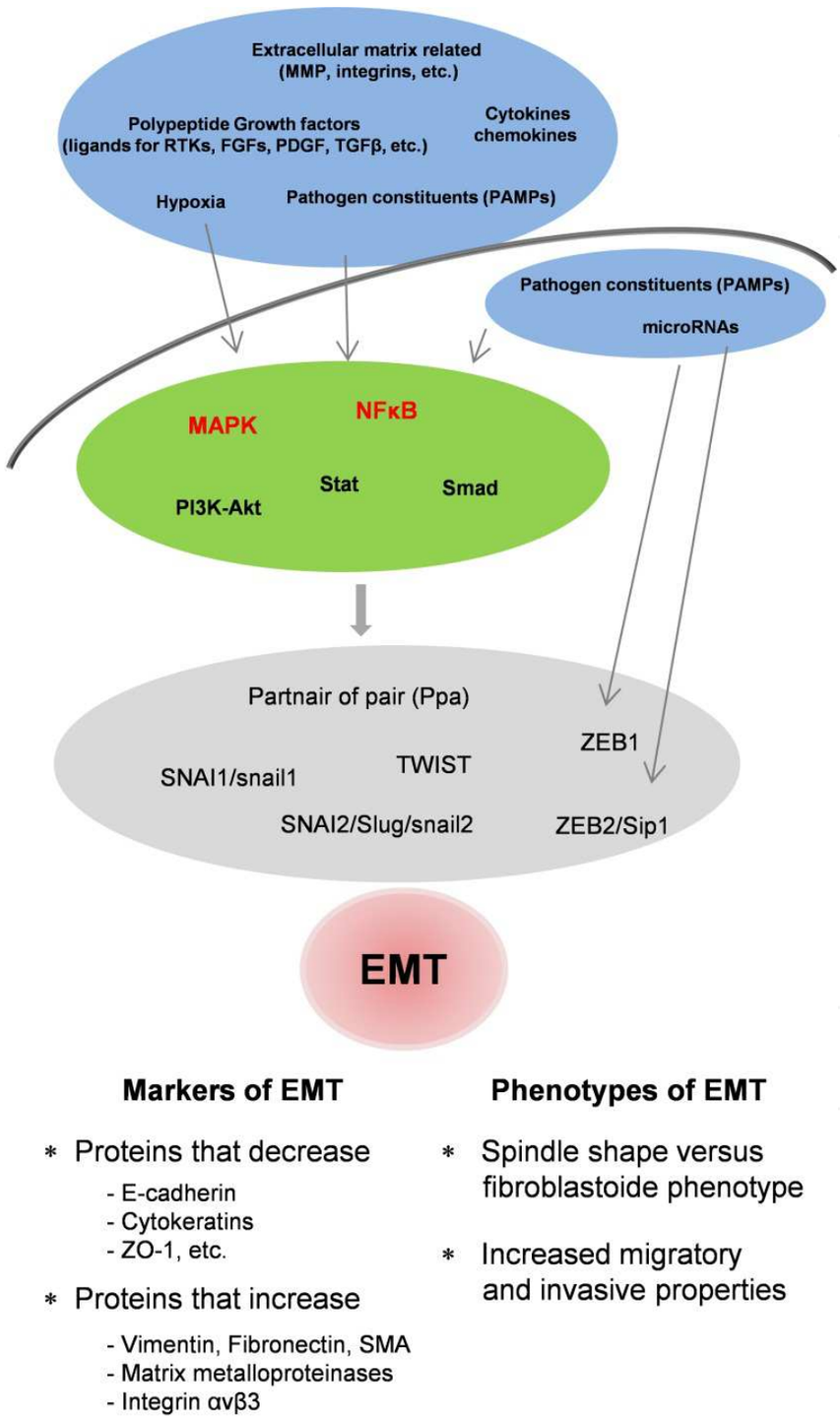

Fig. 1. A basic view of signaling pathways involved in EMT

EMT can result from various extracellular (ligands for RTKs such as FGFs, PDFG; ligands for RS/TKs such as TGFβ or ligands for specific receptors such as Wnts) and cellular stimuli (extracellular matrix compounds, hypoxia, microRNAs, ROS) that through interactions with specific receptors or other mechanisms can stimulate intracellular signaling pathways, leading to activation of transcription factors that govern the transcription of EMT-related genes. The Figure highlights NF-κB and MAPK signaling as major pathways involved in EMT triggering. The most relevant criteria to detect EMT are loss of epithelial markers (E-cadherin, Cytokeratins, ZO-1 and *etc.*) gain of mesenchymal markers (vimentin, fibronectin, MMPs, proteins of the extracellular matrix, *etc.*) associated to appearance of the fibroblastoide phenotype and increased migratory and invasive properties.

2.2 EMT and transcription factors

EMT is controlled by a small group of transcription factors defined as the core EMT regulatory factors that comprises SNAI1/Snail1 (Twigg, S. R. & Wilkie, A. O., 1999), SNAI2/Slug/Snail2 (Cohen, M. E., et al., 1998), Sip1/ZEB2 (Verschueren, K., et al., 1999) and Twist (Wang, S. M., et al., 1997). Whereas these proteins share the same function that is a transcriptional repression of E-cadherin, they have different structures. The Snail family is composed of zinc finger proteins, the ZEB family has 2 zinc finger clusters and Twist proteins has a helix loop helix motif (Peinado, H., et al., 2007). 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) (Lander, R., et al., 2011). 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.

2.3 EMT and intracellular signaling pathways

Multiple signaling pathways, including receptor tyrosine kinase–mediated signals, transforming growth factor (TGF)-β/Smad, Wnt, Notch and hypoxia have been implicated as upstream initiators of the EMT process as highlighted in recent reviews (Moustakas, A. & Heldin, C. H., 2007, Peinado, H., et al., 2007, Said, N. A. & Williams, E. D., 2011). We will focus here on pathways that are activated by pathogen recognition receptors as detailed later in this review.

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 module (MAPKKK → MAPKK → MAPK). This module comprises four different signaling pathways activated by mitogens, inflammation, stress and oxidative stress (Junttila, M. R., et al., 2008). These signaling pathways are interconnected.

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 activation of a set of transcription factors which in turn control many cellular responses that are relevant for EMT (Keshet, Y. & Seger, R., 2010). Indeed, in addition to repress E-cadherin 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 (Grunert, S., et al., 2003) and references herein.

The p38 MAPK pathway can be activated in response to various cytokines, as well as pathogens and by environmental stress such as hypoxia. p38 MAPK was first described to down-regulate E-cadherin expression during mouse gastrulation (Zohn, I. E., et al., 2006). Further, p38 MAPK was described to participate in TNF-α (Grund, E. M., et al., 2008) and TGF-β-induced EMT (Borthwick, L. A., et al., 2011). In addition a crosstalk between the Smad and NR-κB pathways accentuates TGF-β-induced EMT in presence of TNF-α.

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 TGF-β-induced EMT in keratinocytes (Santibanez, J. F., 2006). Further it was shown that activation of Smad3 by JNK is necessary to mediate TGF-β-induced EMT (Liu, Q., et al., 2008).

The Smad pathway

The best described inductor of the Smad pathway is the TGF-β that is widely described as an EMT inductor; for a review see (Zavadil, J. & Bottinger, E. P., 2005). Briefly, TGF-β binds to its receptor which then activates by phosphorylation two transcription factors, Smad-2 and Smad-3 (Massague, J., 1998). 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 (Wu, J. W., et al., 2001). Moreover, TGF-β activates Smad-independent signaling cascade leading to the activation of the classical Ras-MAPK pathway (Said, N. A. & Williams, E. D., 2011).

In addition to its well-known function in tumor progression, the TGF-β signaling plays an essential role in establishing immunological tolerance (Wan, Y. Y. & Flavell, R. A., 2007). Interestingly, reports indicate that microbe invasion lead to TGF-β modulation (Reed, S. G., 1999). First, it was shown, *in vitro* and *in vivo*, that macrophages invasion by *Trypanosoma cruzi* led to production of TGF-β (Silva, J. S., et al., 1991). This observation was then extended to bacterial infections with studies using *Mycobacterium avium* and *Mycobacterium tuberculosis* (Champsi, J., et al., 1995, Toossi, Z., et al., 1995). In the last decade it appears that many bacteria or viruses induce TGF-β production via signaling pathways that require Toll like receptors (TLR) as described below.

Macrophages represent the first line of defense; indeed most of these studies were performed on immune cells. However, TGF-β released by macrophages could activate TGF signaling on epithelial cells and then induce EMT. In agreement with this paracrine loop hypothesis, it has recently been demonstrated that increasing numbers of leukocytes (macrophages and T cells) infiltrating the kidney after acute unilateral ureteral obstruction in a mouse model correlate with increased EMT (Lange-Sperandio, B., et al., 2007).

The STAT pathway

The signal transducers and activators of transcription (STAT) family consist of seven proteins. STATs 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 (Reich, N. C. & Liu, L., 2006). In general, STAT proteins have important roles in the immune response (Ihle, J. N., 2001), however STAT3 has been more particularly involved in EMT. Invalidation of the stat3 gene in mice results in early embryonic lethality (Takeda, K., et al., 1997), therefore using small interference RNA technology to efficiently block STAT3 signaling, Huang and co-authors demonstrated in pancreatic cancer cells that silencing of STAT3 resulted in suppression of EMT (Huang, C., et al., 2011).

Hypoxia

Alteration in microenvironmental oxygen tension and activation of hypoxic signaling through hypoxia-inducible factor (HIF) are emerging as important triggers and modulators of EMT (Haase, V. H., 2009, Jiang, J., et al., 2011). *In vivo*, O_2 tension varies from 2.5% to 9% in most healthy tissues. However, inflamed or diseased tissues can be deprived of O_2 (hypoxia) due to vascular damage, intensive metabolic activity of bacteria and other pathogens, and large numbers of infiltrating cells, leading to O_2 levels of less than 1%. This phenomenon results in activation of the well-coordinated mechanism leading to regulation of HIF transcriptional pathways as described in many reviews such as (Imtiyaz, H. Z., et al., 2010).

Oxygen deprivation is not the only inducer of HIF. Indeed, inflammatory cytokines, growth factors and bacterial products under normoxic conditions also induce HIFs (Blouin, C. C., et al., 2004, Cane, G., et al., 2010, Jung, Y. J., et al., 2003, Peyssonnaux, C., et al., 2008, Zhou, J., et al., 2003). Once activated HIFs, and more particularly HIF-1, controls E-cadherin repression, loss of cell-cell adhesion and cell motility via regulation of the core EMT regulatory factors in various cell types, as described in (Haase, V. H., 2009). For example, HIF-1, regulates the expression of TWIST by binding directly to the hypoxia response element in the TWIST proximal promoter (Yang, M. H., et al., 2008).

NF-κB

The NF-κB family of transcription factors which is composed of five members - p65 (REL-A), REL-B, cytoplasmic (c) REL, p50 and p52 - is widely activated under cytokines and/or microbial challenge (Li, Q. & Verma, I. M., 2002, Min, C., et al., 2008).

For example, the proinflammatory cytokines interleukin-1β and tumor necrosis factor (TNF)-α both activate and are activated by NF-κB, thus creating a positive feedback loop that results in perpetual amplification of the response.

Together with SMADs and HIF-1α, NF-κB has been shown, in an integrative genomic analysis, to regulate ZEB2, an EMT regulator, (Katoh, M., 2009). In addition, NF-κB is involved in the up regulation of *twist-1* and *twist-2* expression in response to TNF-α; this regulation is lost in fibroblasts lacking the p65 subunit of NF-κB (Sosic, D., et al., 2003). 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 NF-κB 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 NF-κB promote a partial reversion to an epithelial phenotype (Pantuck, A. J., et al., 2010).

Finally, NF-κB also controls mesenchymal marker expression. The NF-kB binding site has been described on the vimentin gene (Lilienbaum, A., et al., 1990) and overexpression of a

constitutively active form of p65 in breast cancer cells increases expression of vimentin (Chua, H. L., et al., 2007). Moreover, NF-κB directly activates the transcription of the matrix metalloprotease (MMP)-9 gene, a type IV collagenase which increases cellular invasiveness and motility (Himelstein, B. P., et al., 1997) and indirectly controls MMP-2 (Yoshizaki, T., et al., 2002).

MicroRNAs

MicroRNAs (miRs) are non-coding RNA of 18-24 bp that post transcriptionally regulate gene expression. The key region of miRs that governs their target specificity, named the seed sequence, encompasses bases 2-7 from their 5′ end (Lewis, B. P., et al., 2005). More than 1200 miRNAs have been identified in humans, and each individual miR could regulate ten to hundreds of genes according to the presence of seed sequence matches in their 3′UTRs. The ability of a specific miR to modify gene expression is governed by its seed sequence but also its expression, which could be spatiotemporally regulated.

A microRNA microarray profiling performed on MDCK undergoing EMT allowed to characterize the implication of the miR-200 family (miR-200a, miR-200b, miR-200c, miR-141 and miR-429) and miR-205. Decrease in expression of each of these miRs correlates with decreased expression of E-cadherin and increases in mesenchymal markers mRNA such as vimentin and fibronectin. In addition, overexpression of these miRs in MDCK cells prevents EMT demonstrating that down-regulation of these miRs is an essential component of the EMT process. Finally, it was shown that the miR-200 family represses endogenous expression of ZEB1 and ZEB2 (Bracken, C. P., et al., 2008, Gregory, P. A., et al., 2008, Korpal, M., et al., 2008, Park, S. M., et al., 2008).

As mentioned previously in this report, TGFβ is a powerful inductor of EMT. A combination of miRs and mRNA profiling was used to identify miRs that destabilize mRNAs in TGFβ-directed EMT. Such strategy allowed the characterization of eight miRs specific of a particular signature of EMT-like response (Zavadil, J., et al., 2007).

3. Pattern recognition receptor-induced signaling pathways

3.1 A general overview on PRR

Charles Janeway was the first to understand that recognition of pathogen-associated molecular patterns (PAMPs) by host pathogen-recognition receptors (PRRs) is the basis of immune immunity and represents the first defense against pathogens (Janeway, C. A., Jr., 1989). His discovery was further confirmed by the identification of Toll-like receptor (TLR)4 as the protein involved in the recognition of lipopolysaccharide (LPS), therefore making the link between a microbial motif, LPS, and a host receptor, TLR4 (Poltorak, A., et al., 1998). A new axe of researches was then opened and after more than a decade the TLRs family encounters 10 members in human and each TLR has a distinct function in terms of PAMP recognition (Kawai, T. & Akira, S., 2010).

TLRs are divided into two subgroups based on their cellular localization and respective PAMP ligands. The first group, expressed on cell surfaces which recognize mainly microbial membrane components such as lipids, lipoproteins and proteins, is composed of TLR1, TLR2, TLR4, TLR5, TLR6 and TLR11; 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); NLRs sense the presence of intracellular muropeptides (Fritz, J. H., et al., 2006). As highlighted in **Figure 2** both TLRs and NLRs activate intracellular signaling pathways that share common adaptors with receptors of growth factors, cytokines or chemokines.

Note that in addition to TLRs and NLRs other microbial sensors exist as reviewed in (Bouchon, A., et al., 2000, Crocker, P. R., 2005, Klesney-Tait, J., et al., 2006, Robinson, M. J., et al., 2006).

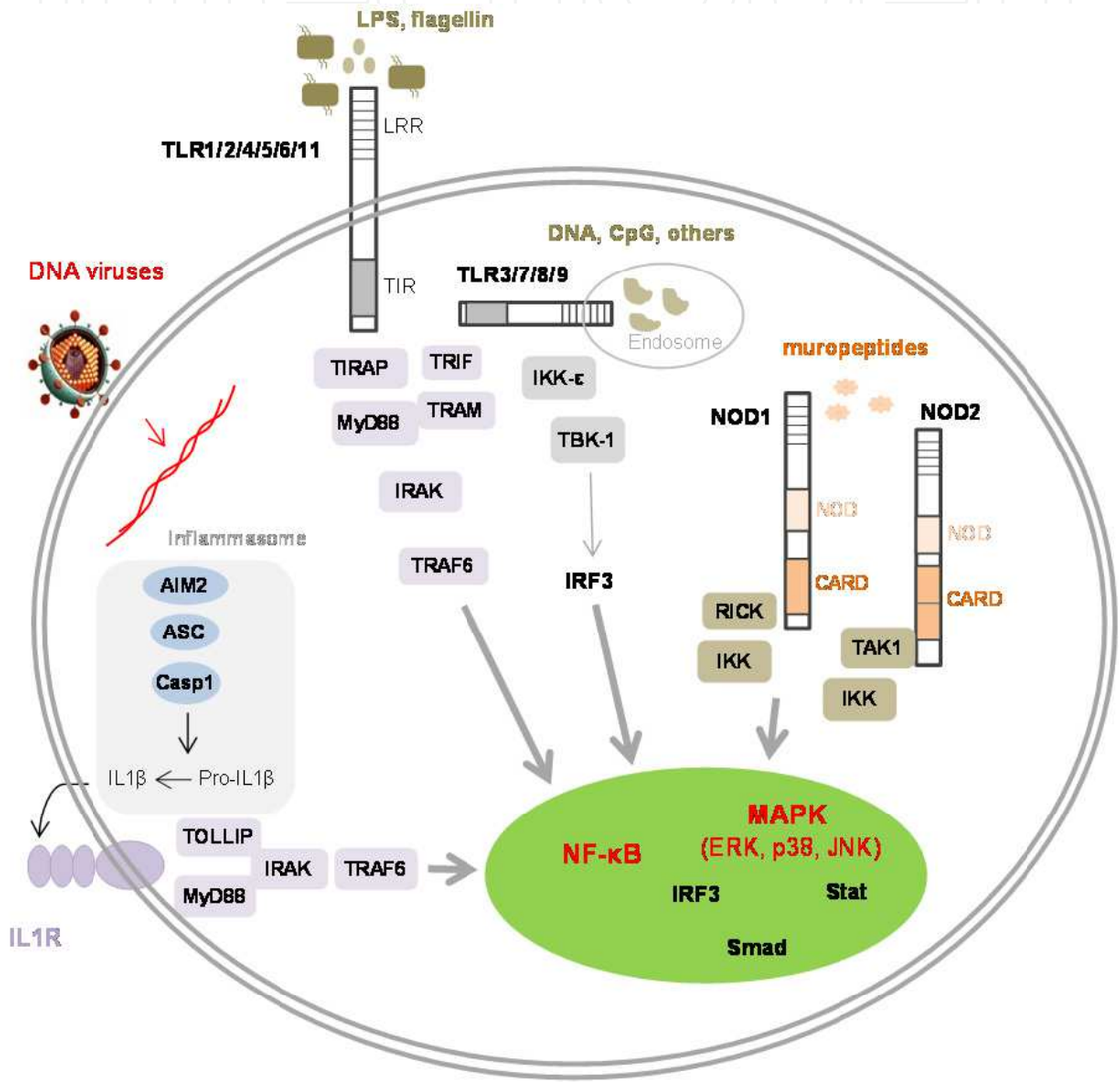

Fig. 2. A schematic view of PPR-induced pathways involved in stimulation of NF-κB and MAPK signaling

TLRs (TLR1, TLR2, TLR4, TLR5, TLR6, TLR7 and TLR9) do activate NF-κB and MAPK module – comprising ERK, p38 and JNK – by binding of MyD88 to the receptor TIR domain and subsequently triggering IRAK, TRAF6 and TAK1 which ultimately activate the IkB kinase (IKK) complex – which consists of IKK-α, IKK-β and IKK-γ (also known as IKK1,

IKK2 and nuclear factor-κB (NF-κB) essential modulator, NEMO, respectively – and MAPKs. Alternatively, TIRAP (TIR domain-containing adaptor protein), a second TIR-domain-containing adaptor protein, is involved in the MyD88-dependent signalling pathway through TLR2 and TLR4. A third TIR-domain-containing adaptor, TRIF (TIR domain-containing adaptor protein inducing IFN-β), is essential for the MyD88-independent pathway. Further, the non-typical IKKs IKK-ε and TBK1 (TRAF-family-member-associated NF-κB 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.

3.2 PRR and mediators of EMT

TLRs are type I trans-membrane proteins with extracellular domains containing leucine-rich repeats and mediating the recognition of PAMPs, trans-membrane domains and 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 NF-κB and MAPK which in turn induces various transcription factors (Kawai, T. & Akira, S., 2010).

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. The final consequence of this signaling puzzle is the activation of NF-κB and MAPK signaling pathways. TRIF is used by TLR3 and TLR4 and induces alternative pathways that lead to activation of the transcription factors IRF3 and NF-κB

Interestingly enough, host recognition of pathogens lead to activation of NF-κB and MAPK pathways. As mentioned earlier, these two pathways are particularly relevant for EMT since they control the activation of transcription factors that in turn regulate the expression of the EMT core genes.

4. EMT and bacterial pathogens

The microbes normally present in humans are collectively estimated to number tenfold that of human cells. Mainly located in the gut, the microbiota is crucial for human life by influencing human physiology and nutriment uptake (Ley, R. E., et al., 2006). In addition, the microbiota contributes to the shaping of healthy intestinal immune responses (Inagaki, H., et al., 1996). 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 (Round, J. L. & Mazmanian, S. K., 2009) 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.

4.1 Lipopolysaccharide

Lipopolysaccharide (LPS) is the major component of the outer membrane of Gram-negative bacteria. 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 (Zhao, L., et al., 2010). 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 TGF-β1/Smad2/3 pathway.

4.2 *Helicobacter pylori*

Helicobacter pylori is a gram-negative bacteria which colonizes the human stomach of about 50% of the world's population. Although a large proportion of infected subjects can develop gastritis, 80% of these individuals remain asymptomatic. Severe *H. pylori*-mediated diseases are duodenal and gastric ulcer disease, gastric cancer and mucosa-associated lymphoid tissues (MALT) lymphomas affecting about 15%, 1% and 0.1% of infected people, respectively (Amieva, M. R. & El-Omar, E. M., 2008). Since 1994, *H. pylori* is classified as a class I carcinogen by the World Health Organization. More than 350 genetically different strains have been identified. To avoid mechanical clearance, *H. pylori* first adhere to the gastric epithelium due to adhesins. Among their numerous virulence factors, the two major virulence factors of *H. pylori*, the cytotoxin VacA and the cag pathogenicity island and its effector CagA, can co-opt epithelial cell function. Whereas VacA can disrupt the barrier function of tight junction, it does not perturb junction integrity (Papini, E., et al., 1998), CagA has major effects on the apical junctional complex allowing the deregulation of epithelial cell-cell adhesion and a loss in epithelial polarity (Amieva, M. R., et al., 2003, Murata-Kamiya, N., et al., 2007).

Using the pathogenic H. Pylori strain 60190, Yin and co-authors observed expression of Snail and Slug in gastric epithelial cells (Yin, Y., et al., 2010). 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>NF-κb signaling pathway (Brandt, S., et al., 2005). Then, it appears that NF-κB is a central common effector that plays a key role in the EMT process.

As mentioned earlier, HIFs are also involved in EMT regulation. It is noteworthy that ROS stabilize HIF-1α (Park, J. H., et al., 2003) and *H. pylori* induce ROS (Bagchi, D., et al., 1996).

Therefore, one could speculate that *H. pylori*-induced stabilization of HIF-1 acts in combination with NF-κB to maximally induce the EMT program.

4.3 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-, enterohaemorrhagic-, enterotoxigenic-, enteroaggregative-, enteroinvasive- and diffusely adherent-*E. coli*. Enteropathogenic *E. coli* cause entero/diarrhoeal disease as a consequence of lack of intestinal barrier permeability (Kaper, J. B., et al., 2004). 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 (Servin, A. L., 2005) The pathogenicity of such bacteria is still controversial; however, the presence of DAEC expressing Afa/Dr adhesins has been reported in epidemiological studies of various types of enterocolitis (Meraz, I. M., et al., 2007, Vargas, M., et al., 1998). Afa/Dr DAEC strains are a family of DAEC expressing the afimbrial Afa-I and Afa-III adhesins, Dr haemagglutinin and fimbrial F1845 adhesin. Afa/Dr adhesins interact with receptors such as the membrane-associated decay accelerating factor (DAF/ CD55), the carcino-embryonic-antigen (CEA/CD66e) and CEACAM-1, -3, -6 (Berger, C. N., et al., 2004), leading to cell signaling. Using the clinical isolate DAEC C1845, we have shown that infection of intestinal epithelial cells promotes 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 (Betis, F., et al., 2003a, 2003b, Cane, G., et al., 2007). 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 (Cane, G., et al., 2010), therefore highlighting the critical role of HIF in DAEC-induced EMT.

5. EMT and viral pathogens

As for microbial pathogens, viral infection leads to activation of intracellular signaling pathways (Rathinam, V. A. & Fitzgerald, K. A., 2011); 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's 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.

5.1 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. Beyond immune cells, EBV also infects epithelial cells and it has been associated with neoplastic diseases such as nasopharyngeal carcinoma (Chen, M.-R., 2011); 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 (Caldwell, R. G., et al., 1998, Gires, O., et al., 1997), 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 (Horikawa, T., et al., 2007). 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 NF-κB in nasopharyngeal epithelial cells. More recently the same group demonstrated that Snail1 acts in combination to twist1 to induce EMT in nasopharyngeal carcinoma cells (Horikawa, T., et al., 2011).

Using nasopharyngeal carcinoma tumor samples the group of Zeng has shown that 57.6% of tumors overexpressed LMP2A at the tumor invasive front (Kong, Q. L., et al., 2010). Interestingly enough, LMP2A increases the size of the stem-like cell population and the number of tumor initial cells; this effect being reversed by inhibitors of AKT.

In addition to a classical effect on intracellular signaling, EBV also down regulates expression of miR-200a and miR-200b, the down regulation of which induces EMT (Shinozaki, A., et al., 2010). First, the authors demonstrated an association between miR-200a and miR-200b down regulation and E-cadherin expression on resected gastric carcinoma tissue. Further, using *in vitro* established EBV-infected cell lines they confirmed that down regulation of these miRs correlates with up regulation 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 down regulate the pri-miR-200 transcript.

EBV is found in alveolar epithelial cells where it is suspected to promote idiopathic pulmonary fibrosis. Indeed, active EVB infection regulates EMT in alveolar epithelial cells (Malizia, A. P., et al., 2009). In this report the authors highlighted the role of Wnt signaling, since Wnt5B-silenced cells are resistant to EBV-induced EMT. Further, using an *ex vivo* cell system model the authors demonstrated that activation of non-canonical Wnt signaling pathway by EBV is dependent of CUX1 signaling. Therefore a link between EBV and fibrosis was demonstrated with EMT being the core of the process. This former observation was recently confirmed and extended. Indeed, the group of Lasky demonstrated that LMP1 induces pro-EMT signaling that occurs primarily through the nuclear factor-κB pathway and secondarily through the extracellular signal-regulated kinase (ERK) pathway (Sides, M. D., et al., 2011).

5.2 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 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 (Yang, S. Z., et al., 2009). 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 (Delhem, N., et al., 2001, Lai, M. M. & Ware, C. F., 2000, Zhu, N., et al., 1998). Of particular interest, HCV core protein interacts with Smad3 and consequently inhibits TGF-β induced Smad3 transcriptional activity (Pavio, N., et al., 2005). Since the TGF-β/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 TGF-β I receptor thus demonstrating a TGF-β dependent effect of core on EMT development (Battaglia, S., et al., 2009).

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 (Li, T., et al., 2010).

6. 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, as shown in **Figure 3**, we have attempted to depict the main lines which 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. 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, cancer) (Hofman, P. M., 2010) have been described to induce EMT. Given that all pathogen recognition receptors induce NF-κB and MAPK module, one can speculate

that each pathogen may have the potential to induce EMT as for 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.

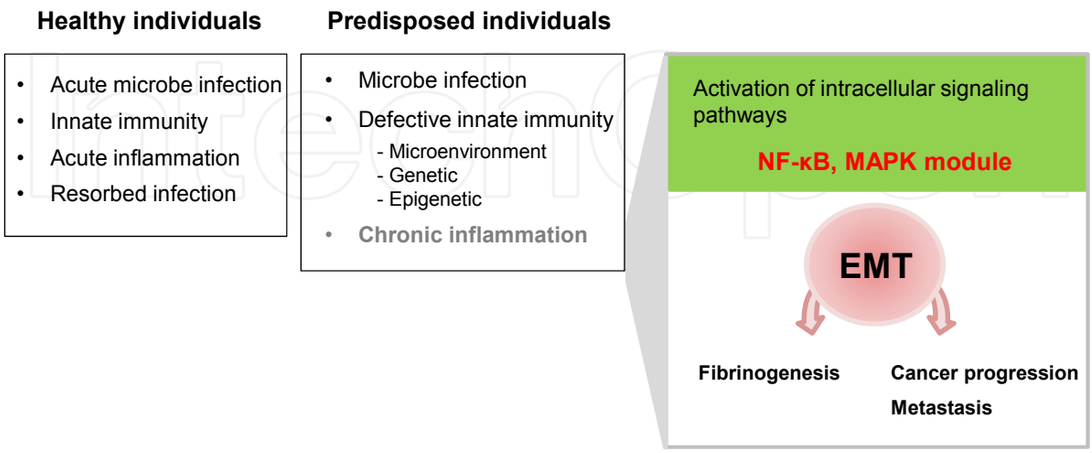

Fig. 3. Microbe-induced chronic inflammation in predisposed individuals leads to EMT

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 NF-κB 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.

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8. References

M. G. Farquhar & G. E. Palade (1963). Junctional complexes in various epithelia, *J Cell Biol,* Vol.17, pp.375-412, ISSN 0021-9525

S. Grunert, M. Jechlinger & H. Beug (2003). Diverse cellular and molecular mechanisms contribute to epithelial plasticity and metastasis, *Nat Rev Mol Cell Biol,* Vol.4, No.8, pp.657-65, ISSN 1471-0072

J. P. Thiery (2002). Epithelial-mesenchymal transitions in tumour progression, *Nat Rev Cancer,* Vol.2, No.6, pp.442-54, ISSN 1474-175X

O. Ilina & P. Friedl (2009). Mechanisms of collective cell migration at a glance, *J Cell Sci,* Vol.122, No.Pt 18, pp.3203-8, ISSN 1477-9137

M. A. Nieto (2011). the ins and outs of the epithelial to mesenchymal transition in health and disease, *Annual review Cell Dev Biol,* Vol.in press

J. P. Thiery, H. Acloque, R. Y. Huang & M. A. Nieto (2009). Epithelial-mesenchymal transitions in development and disease, *Cell,* Vol.139, No.5, pp.871-90, ISSN 1097-4172

S. R. Twigg & A. O. Wilkie (1999). Characterisation of the human snail (SNAI1) gene and exclusion as a major disease gene in craniosynostosis, *Hum Genet,* Vol.105, No.4, pp.320-6, ISSN 0340-6717

M. E. Cohen, M. Yin, W. A. Paznekas, M. Schertzer, S. Wood & E. W. Jabs (1998). Human SLUG gene organization, expression, and chromosome map location on 8q, *Genomics,* Vol.51, No.3, pp.468-71, ISSN 0888-7543

K. Verschueren, J. E. Remacle, C. Collart, H. Kraft, B. S. Baker, P. Tylzanowski, L. Nelles, G. Wuytens, M. T. Su, R. Bodmer, J. C. Smith & D. Huylebroeck (1999). SIP1, a novel zinc finger/homeodomain repressor, interacts with Smad proteins and binds to 5'-CACCT sequences in candidate target genes, *J Biol Chem,* Vol.274, No.29, pp.20489-98, ISSN 0021-9258

S. M. Wang, V. W. Coljee, R. J. Pignolo, M. O. Rotenberg, V. J. Cristofalo & F. Sierra (1997). Cloning of the human twist gene: its expression is retained in adult mesodermally-derived tissues, *Gene,* Vol.187, No.1, pp.83-92, ISSN 0378-1119

H. Peinado, D. Olmeda & A. Cano (2007). Snail, Zeb and bHLH factors in tumour progression: an alliance against the epithelial phenotype?, *Nat Rev Cancer,* Vol.7, No.6, pp.415-28, ISSN 1474-175X

R. Lander, K. Nordin & C. Labonne (2011). The F-box protein Ppa is a common regulator of core EMT factors Twist, Snail, Slug, and Sip1, *J Cell Biol,* Vol.194, No.1, pp.17-25, ISSN 1540-8140

A. Moustakas & C. H. Heldin (2007). Signaling networks guiding epithelial-mesenchymal transitions during embryogenesis and cancer progression, *Cancer Sci,* Vol.98, No.10, pp.1512-20, ISSN 1347-9032

N. A. Said & E. D. Williams (2011). Growth factors in induction of epithelial-mesenchymal transition and metastasis, *Cells Tissues Organs,* Vol.193, No.1-2, pp.85-97, ISSN 1422-6421

M. R. Junttila, S. P. Li & J. Westermarck (2008). Phosphatase-mediated crosstalk between MAPK signaling pathways in the regulation of cell survival, *FASEB J,* Vol.22, No.4, pp.954-65, ISSN 1530-6860

Y. Keshet & R. Seger (2010). The MAP kinase signaling cascades: a system of hundreds of components regulates a diverse array of physiological functions, *Methods Mol Biol,* Vol.661, pp.3-38, ISSN 1940-6029

I. E. Zohn, Y. Li, E. Y. Skolnik, K. V. Anderson, J. Han & L. Niswander (2006). p38 and a p38-interacting protein are critical for downregulation of E-cadherin during mouse gastrulation, *Cell,* Vol.125, No.5, pp.957-69, ISSN 0092-8674

E. M. Grund, D. Kagan, C. A. Tran, A. Zeitvogel, A. Starzinski-Powitz, S. Nataraja & S. S. Palmer (2008). Tumor necrosis factor-alpha regulates inflammatory and mesenchymal responses via mitogen-activated protein kinase kinase, p38, and nuclear factor kappaB in human endometriotic epithelial cells, *Mol Pharmacol,* Vol.73, No.5, pp.1394-404, ISSN 1521-0111

L. A. Borthwick, A. Gardner, A. De Soyza, D. A. Mann & A. J. Fisher (2011). Transforming Growth Factor-beta1 (TGF-beta1) Driven Epithelial to Mesenchymal Transition (EMT) is Accentuated by Tumour Necrosis Factor alpha (TNFalpha) via Crosstalk Between the SMAD and NF-kappaB Pathways, *Cancer Microenviron,* ISSN 1875-2284 J. F. Santibanez (2006). JNK mediates TGF-beta1-induced epithelial mesenchymal transdifferentiation of mouse transformed keratinocytes, *FEBS Lett,* Vol.580, No.22, pp.5385-91, ISSN 0014-5793

Q. Liu, H. Mao, J. Nie, W. Chen, Q. Yang, X. Dong & X. Yu (2008). Transforming growth factor {beta}1 induces epithelial-mesenchymal transition by activating the JNK-Smad3 pathway in rat peritoneal mesothelial cells, *Perit Dial Int,* Vol.28 Suppl 3, pp.S88-95, ISSN 0896-8608

J. Zavadil & E. P. Bottinger (2005). TGF-beta and epithelial-to-mesenchymal transitions, *Oncogene,* Vol.24, No.37, pp.5764-74, ISSN 0950-9232

J. Massague (1998). TGF-beta signal transduction, *Annu Rev Biochem,* Vol.67, pp.753-91, ISSN 0066-4154

J. W. Wu, M. Hu, J. Chai, J. Seoane, M. Huse, C. Li, D. J. Rigotti, S. Kyin, T. W. Muir, R. Fairman, J. Massague & Y. Shi (2001). Crystal structure of a phosphorylated Smad2. Recognition of phosphoserine by the MH2 domain and insights on Smad function in TGF-beta signaling, *Mol Cell,* Vol.8, No.6, pp.1277-89, ISSN 1097-2765

Y. Y. Wan & R. A. Flavell (2007). 'Yin-Yang' functions of transforming growth factor-beta and T regulatory cells in immune regulation, *Immunol Rev,* Vol.220, pp.199-213, ISSN 0105-2896 S. G. Reed (1999). TGF-beta in infections and infectious diseases, *Microbes Infect,* Vol.1, No.15, pp.1313-25, ISSN 1286-4579

J. S. Silva, D. R. Twardzik & S. G. Reed (1991). Regulation of Trypanosoma cruzi infections in vitro and in vivo by transforming growth factor beta (TGF-beta), *J Exp Med,* Vol.174, No.3, pp.539-45, ISSN 0022-1007

J. Champsi, L. S. Young & L. E. Bermudez (1995). Production of TNF-alpha, IL-6 and TGF-beta, and expression of receptors for TNF-alpha and IL-6, during murine Mycobacterium avium infection, *Immunology,* Vol.84, No.4, pp.549-54, ISSN 0019-2805

Z. Toossi, T. G. Young, L. E. Averill, B. D. Hamilton, H. Shiratsuchi & J. J. Ellner (1995). Induction of transforming growth factor beta 1 by purified protein derivative of Mycobacterium tuberculosis, *Infect Immun,* Vol.63, No.1, pp.224-8, ISSN 0019-9567

B. Lange-Sperandio, A. Trautmann, O. Eickelberg, A. Jayachandran, S. Oberle, F. Schmidutz, B. Rodenbeck, M. Homme, R. Horuk & F. Schaefer (2007). Leukocytes induce epithelial to mesenchymal transition after unilateral ureteral obstruction in neonatal mice, *Am J Pathol,* Vol.171, No.3, pp.861-71, ISSN 0002-9440

N. C. Reich & L. Liu (2006). Tracking STAT nuclear traffic, *Nat Rev Immunol,* Vol.6, No.8, pp.602-12, ISSN 1474-1733 (Print)

1474-1733 (Linking)

J. N. Ihle (2001). The Stat family in cytokine signaling, *Curr Opin Cell Biol,* Vol.13, No.2, pp.211-7, ISSN 0955-0674

K. Takeda, K. Noguchi, W. Shi, T. Tanaka, M. Matsumoto, N. Yoshida, T. Kishimoto & S. Akira (1997). Targeted disruption of the mouse Stat3 gene leads to early embryonic lethality, *Proc Natl Acad Sci U S A,* Vol.94, No.8, pp.3801-4, ISSN 0027-8424

C. Huang, G. Yang, T. Jiang, G. Zhu, H. Li & Z. Qiu (2011). The effects and mechanisms of blockage of STAT3 signaling pathway on IL-6 inducing EMT in human pancreatic cancer cells in vitro, *Neoplasma,* Vol.58, No.5, pp.396-405, ISSN 0028-2685

V. H. Haase (2009). Oxygen regulates epithelial-to-mesenchymal transition: insights into molecular mechanisms and relevance to disease, *Kidney Int,* Vol.76, No.5, pp.492-9, ISSN 1523-1755

J. Jiang, Y. L. Tang & X. H. Liang (2011). EMT: a new vision of hypoxia promoting cancer progression, *Cancer Biol Ther,* Vol.11, No.8, pp.714-23, ISSN 1555-8576

H. Z. Imtiyaz, E. P. Williams, M. M. Hickey, S. A. Patel, A. C. Durham, L. J. Yuan, R. Hammond, P. A. Gimotty, B. Keith & M. C. Simon (2010). Hypoxia-inducible factor 2alpha regulates macrophage function in mouse models of acute and tumor inflammation, *J Clin Invest,* Vol.120, No.8, pp.2699-714, ISSN 1558-8238

C. C. Blouin, E. L. Page, G. M. Soucy & D. E. Richard (2004). Hypoxic gene activation by lipopolysaccharide in macrophages: implication of hypoxia-inducible factor 1alpha, *Blood,* Vol.103, No.3, pp.1124-30, ISSN 0006-4971

G. Cane, A. Ginouves, S. Marchetti, R. Busca, J. Pouyssegur, E. Berra, P. Hofman & V. Vouret-Craviari (2010). HIF-1alpha mediates the induction of IL-8 and VEGF expression on infection with Afa/Dr diffusely adhering E. coli and promotes EMT-like behaviour, *Cell Microbiol,* Vol.12, No.5, pp.640-53, ISSN 1462-5822

Y. J. Jung, J. S. Isaacs, S. Lee, J. Trepel & L. Neckers (2003). IL-1beta-mediated up-regulation of HIF-1alpha via an NFkappaB/COX-2 pathway identifies HIF-1 as a critical link between inflammation and oncogenesis, *FASEB J,* Vol.17, No.14, pp.2115-7, ISSN 1530-6860

C. Peyssonnaux, A. T. Boutin, A. S. Zinkernagel, V. Datta, V. Nizet & R. S. Johnson (2008). Critical role of HIF-1alpha in keratinocyte defense against bacterial infection, *J Invest Dermatol,* Vol.128, No.8, pp.1964-8, ISSN 1523-1747

J. Zhou, T. Schmid & B. Brune (2003). Tumor necrosis factor-alpha causes accumulation of a ubiquitinated form of hypoxia inducible factor-1alpha through a nuclear factor-kappaB-dependent pathway, *Mol Biol Cell,* Vol.14, No.6, pp.2216-25, ISSN 1059-1524

M. H. Yang, M. Z. Wu, S. H. Chiou, P. M. Chen, S. Y. Chang, C. J. Liu, S. C. Teng & K. J. Wu (2008). Direct regulation of TWIST by HIF-1alpha promotes metastasis, *Nat Cell Biol,* Vol.10, No.3, pp.295-305, ISSN 1476-4679

Q. Li & I. M. Verma (2002). NF-kappaB regulation in the immune system, *Nat Rev Immunol,* Vol.2, No.10, pp.725-34, ISSN 1474-1733

C. Min, S. F. Eddy, D. H. Sherr & G. E. Sonenshein (2008). NF-kappaB and epithelial to mesenchymal transition of cancer, *J Cell Biochem,* Vol.104, No.3, pp.733-44, ISSN 1097-4644

M. Katoh (2009). Integrative genomic analyses of ZEB2: Transcriptional regulation of ZEB2 based on SMADs, ETS1, HIF1alpha, POU/OCT, and NF-kappaB, *Int J Oncol,* Vol.34, No.6, pp.1737-42, ISSN 1019-6439

D. Sosic, J. A. Richardson, K. Yu, D. M. Ornitz & E. N. Olson (2003). Twist regulates cytokine gene expression through a negative feedback loop that represses NF-kappaB activity, *Cell,* Vol.112, No.2, pp.169-80, ISSN 0092-8674

A. J. Pantuck, J. An, H. Liu & M. B. Rettig (2010). NF-kappaB-dependent plasticity of the epithelial to mesenchymal transition induced by Von Hippel-Lindau inactivation in renal cell carcinomas, *Cancer Res,* Vol.70, No.2, pp.752-61, ISSN 1538-7445

A. Lilienbaum, M. Duc Dodon, C. Alexandre, L. Gazzolo & D. Paulin (1990). Effect of human T-cell leukemia virus type I tax protein on activation of the human vimentin gene, *J Virol,* Vol.64, No.1, pp.256-63, ISSN 0022-538X

H. L. Chua, P. Bhat-Nakshatri, S. E. Clare, A. Morimiya, S. Badve & H. Nakshatri (2007). NF-kappaB represses E-cadherin expression and enhances epithelial to mesenchymal transition of mammary epithelial cells: potential involvement of ZEB-1 and ZEB-2, *Oncogene,* Vol.26, No.5, pp.711-24, ISSN 0950-9232

B. P. Himelstein, E. J. Lee, H. Sato, M. Seiki & R. J. Muschel (1997). Transcriptional activation of the matrix metalloproteinase-9 gene in an H-ras and v-myc transformed rat embryo cell line, *Oncogene,* Vol.14, No.16, pp.1995-8, ISSN 0950-9232

T. Yoshizaki, H. Sato & M. Furukawa (2002). Recent advances in the regulation of matrix metalloproteinase 2 activation: from basic research to clinical implication (Review), *Oncol Rep,* Vol.9, No.3, pp.607-11, ISSN 1021-335X

B. P. Lewis, C. B. Burge & D. P. Bartel (2005). Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets, *Cell,* Vol.120, No.1, pp.15-20, ISSN 0092-8674

C. P. Bracken, P. A. Gregory, N. Kolesnikoff, A. G. Bert, J. Wang, M. F. Shannon & G. J. Goodall (2008). A double-negative feedback loop between ZEB1-SIP1 and the microRNA-200 family regulates epithelial-mesenchymal transition, *Cancer Res,* Vol.68, No.19, pp.7846-54, ISSN 1538-7445

P. A. Gregory, A. G. Bert, E. L. Paterson, S. C. Barry, A. Tsykin, G. Farshid, M. A. Vadas, Y. Khew-Goodall & G. J. Goodall (2008). The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1, *Nat Cell Biol,* Vol.10, No.5, pp.593-601, ISSN 1476-4679

M. Korpal, E. S. Lee, G. Hu & Y. Kang (2008). The miR-200 family inhibits epithelial-mesenchymal transition and cancer cell migration by direct targeting of E-cadherin transcriptional repressors ZEB1 and ZEB2, *J Biol Chem,* Vol.283, No.22, pp.14910-4, ISSN 0021-9258

S. M. Park, A. B. Gaur, E. Lengyel & M. E. Peter (2008). The miR-200 family determines the epithelial phenotype of cancer cells by targeting the E-cadherin repressors ZEB1 and ZEB2, *Genes Dev,* Vol.22, No.7, pp.894-907, ISSN 0890-9369

J. Zavadil, M. Narasimhan, M. Blumenberg & R. J. Schneider (2007). Transforming growth factor-beta and microRNA:mRNA regulatory networks in epithelial plasticity, *Cells Tissues Organs,* Vol.185, No.1-3, pp.157-61, ISSN 1422-6421

C. A. Janeway, Jr. (1989). Approaching the asymptote? Evolution and revolution in immunology, *Cold Spring Harb Symp Quant Biol,* Vol.54 Pt 1, pp.1-13, ISSN 0091-7451

A. Poltorak, X. He, I. Smirnova, M. Y. Liu, C. Van Huffel, X. Du, D. Birdwell, E. Alejos, M. Silva, C. Galanos, M. Freudenberg, P. Ricciardi-Castagnoli, B. Layton & B. Beutler (1998). Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene, *Science,* Vol.282, No.5396, pp.2085-8, ISSN 0036-8075

T. Kawai & S. Akira (2010). The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors, *Nat Immunol,* Vol.11, No.5, pp.373-84, ISSN 1529-2916

J. H. Fritz, R. L. Ferrero, D. J. Philpott & S. E. Girardin (2006). Nod-like proteins in immunity, inflammation and disease, *Nat Immunol,* Vol.7, No.12, pp.1250-7, ISSN 1529-2908

A. Bouchon, J. Dietrich & M. Colonna (2000). Cutting edge: inflammatory responses can be triggered by TREM-1, a novel receptor expressed on neutrophils and monocytes, *J Immunol,* Vol.164, No.10, pp.4991-5, ISSN 0022-1767

P. R. Crocker (2005). Siglecs in innate immunity, *Curr Opin Pharmacol,* Vol.5, No.4, pp.431-7, ISSN 1471-4892

J. Klesney-Tait, I. R. Turnbull & M. Colonna (2006). The TREM receptor family and signal integration, *Nat Immunol,* Vol.7, No.12, pp.1266-73, ISSN 1529-2908

M. J. Robinson, D. Sancho, E. C. Slack, S. LeibundGut-Landmann & C. Reis e Sousa (2006). Myeloid C-type lectins in innate immunity, *Nat Immunol,* Vol.7, No.12, pp.1258-65, ISSN 1529-2908

R. E. Ley, D. A. Peterson & J. I. Gordon (2006). Ecological and evolutionary forces shaping microbial diversity in the human intestine, *Cell,* Vol.124, No.4, pp.837-48, ISSN 0092-8674

H. Inagaki, T. Suzuki, K. Nomoto & Y. Yoshikai (1996). Increased susceptibility to primary infection with Listeria monocytogenes in germfree mice may be due to lack of accumulation of L-selectin+ CD44+ T cells in sites of inflammation, *Infect Immun,* Vol.64, No.8, pp.3280-7, ISSN 0019-9567

J. L. Round & S. K. Mazmanian (2009). The gut microbiota shapes intestinal immune responses during health and disease, *Nat Rev Immunol,* Vol.9, No.5, pp.313-23, ISSN 1474-1741

L. Zhao, R. Yang, L. Cheng, M. Wang, Y. Jiang & S. Wang (2010). LPS-Induced Epithelial-Mesenchymal Transition of Intrahepatic Biliary Epithelial Cells, *J Surg Res,* ISSN 1095-8673

M. R. Amieva & E. M. El-Omar (2008). Host-bacterial interactions in Helicobacter pylori infection, *Gastroenterology,* Vol.134, No.1, pp.306-23, ISSN 1528-0012

E. Papini, B. Satin, N. Norais, M. de Bernard, J. L. Telford, R. Rappuoli & C. Montecucco (1998). Selective increase of the permeability of polarized epithelial cell monolayers by Helicobacter pylori vacuolating toxin, *J Clin Invest,* Vol.102, No.4, pp.813-20, ISSN 0021-9738

M. R. Amieva, R. Vogelmann, A. Covacci, L. S. Tompkins, W. J. Nelson & S. Falkow (2003). Disruption of the epithelial apical-junctional complex by Helicobacter pylori CagA, *Science,* Vol.300, No.5624, pp.1430-4, ISSN 1095-9203

N. Murata-Kamiya, Y. Kurashima, Y. Teishikata, Y. Yamahashi, Y. Saito, H. Higashi, H. Aburatani, T. Akiyama, R. M. Peek, Jr., T. Azuma & M. Hatakeyama (2007). Helicobacter pylori CagA interacts with E-cadherin and deregulates the beta-catenin signal that promotes intestinal transdifferentiation in gastric epithelial cells, *Oncogene,* Vol.26, No.32, pp.4617-26, ISSN 0950-9232

Y. Yin, A. M. Grabowska, P. A. Clarke, E. Whelband, K. Robinson, R. H. Argent, A. Tobias, R. Kumari, J. C. Atherton & S. A. Watson (2010). Helicobacter pylori potentiates epithelial:mesenchymal transition in gastric cancer: links to soluble HB-EGF, gastrin and matrix metalloproteinase-7, *Gut,* Vol.59, No.8, pp.1037-45, ISSN 1468-3288

S. Brandt, T. Kwok, R. Hartig, W. Konig & S. Backert (2005). NF-kappaB activation and potentiation of proinflammatory responses by the Helicobacter pylori CagA protein, *Proc Natl Acad Sci U S A,* Vol.102, No.26, pp.9300-5, ISSN 0027-8424

J. H. Park, T. Y. Kim, H. S. Jong, Y. S. Chun, J. W. Park, C. T. Lee, H. C. Jung, N. K. Kim & Y. J. Bang (2003). Gastric epithelial reactive oxygen species prevent normoxic degradation of hypoxia-inducible factor-1alpha in gastric cancer cells, *Clin Cancer Res,* Vol.9, No.1, pp.433-40, ISSN 1078-0432

D. Bagchi, G. Bhattacharya & S. J. Stohs (1996). Production of reactive oxygen species by gastric cells in association with Helicobacter pylori, *Free Radic Res,* Vol.24, No.6, pp.439-50, ISSN 1071-5762

J. B. Kaper, J. P. Nataro & H. L. Mobley (2004). Pathogenic Escherichia coli, *Nat Rev Microbiol,* Vol.2, No.2, pp.123-40, ISSN 1740-1526

A. L. Servin (2005). Pathogenesis of Afa/Dr diffusely adhering Escherichia coli, *Clin Microbiol Rev,* Vol.18, No.2, pp.264-92, ISSN 0893-8512

I. M. Meraz, K. Arikawa, H. Nakamura, J. Ogasawara, A. Hase & Y. Nishikawa (2007). Association of IL-8-inducing strains of diffusely adherent Escherichia coli with sporadic diarrheal patients with less than 5 years of age, *Braz J Infect Dis,* Vol.11, No.1, pp.44-9, ISSN 1413-8670

M. Vargas, J. Gascon, F. Gallardo, M. T. Jimenez De Anta & J. Vila (1998). Prevalence of diarrheagenic Escherichia coli strains detected by PCR in patients with travelers' diarrhea, *Clin Microbiol Infect,* Vol.4, No.12, pp.682-688, ISSN 1469-0691

C. N. Berger, O. Billker, T. F. Meyer, A. L. Servin & I. Kansau (2004). Differential recognition of members of the carcinoembryonic antigen family by Afa/Dr adhesins of diffusely adhering Escherichia coli (Afa/Dr DAEC), *Mol Microbiol,* Vol.52, No.4, pp.963-83, ISSN 0950-382X

F. Betis, P. Brest, V. Hofman, J. Guignot, M. F. Bernet-Camard, B. Rossi, A. Servin & P. Hofman (2003a). The Afa/Dr adhesins of diffusely adhering Escherichia coli stimulate interleukin-8 secretion, activate mitogen-activated protein kinases, and promote polymorphonuclear transepithelial migration in T84 polarized epithelial cells, *Infect Immun,* Vol.71, No.3, pp.1068-74, ISSN 0019-9567

F. Betis, P. Brest, V. Hofman, J. Guignot, I. Kansau, B. Rossi, A. Servin & P. Hofman (2003b). Afa/Dr diffusely adhering Escherichia coli infection in T84 cell monolayers induces increased neutrophil transepithelial migration, which in turn promotes cytokine-dependent upregulation of decay-accelerating factor (CD55), the receptor for Afa/Dr adhesins, *Infect Immun,* Vol.71, No.4, pp.1774-83, ISSN 0019-9567

G. Cane, V. L. Moal, G. Pages, A. L. Servin, P. Hofman & V. Vouret-Craviari (2007). Up-regulation of intestinal vascular endothelial growth factor by Afa/Dr diffusely adhering Escherichia coli, *PLoS One,* Vol.2, No.12, pp.e1359, ISSN 1932-6203

V. A. Rathinam & K. A. Fitzgerald (2011). Innate immune sensing of DNA viruses, *Virology,* Vol.411, No.2, pp.153-62, ISSN 1096-0341

M.-R. Chen (2011). Epstein-Barr virus, the immune system, and associated diseases, *Front. Microbio.,* Vol.2:5, ISSN

R. G. Caldwell, J. B. Wilson, S. J. Anderson & R. Longnecker (1998). Epstein-Barr virus LMP2A drives B cell development and survival in the absence of normal B cell receptor signals, *Immunity,* Vol.9, No.3, pp.405-11, ISSN 1074-7613

O. Gires, U. Zimber-Strobl, R. Gonnella, M. Ueffing, G. Marschall, R. Zeidler, D. Pich & W. Hammerschmidt (1997). Latent membrane protein 1 of Epstein-Barr virus mimics a constitutively active receptor molecule, *EMBO J,* Vol.16, No.20, pp.6131-40, ISSN 0261-4189

T. Horikawa, J. Yang, S. Kondo, T. Yoshizaki, I. Joab, M. Furukawa & J. S. Pagano (2007). Twist and epithelial-mesenchymal transition are induced by the EBV oncoprotein latent membrane protein 1 and are associated with metastatic nasopharyngeal carcinoma, *Cancer Res,* Vol.67, No.5, pp.1970-8, ISSN 0008-5472

T. Horikawa, T. Yoshizaki, S. Kondo, M. Furukawa, Y. Kaizaki & J. S. Pagano (2011). Epstein-Barr Virus latent membrane protein 1 induces Snail and epithelial-mesenchymal transition in metastatic nasopharyngeal carcinoma, *Br J Cancer,* Vol.104, No.7, pp.1160-7, ISSN 1532-1827

Q. L. Kong, L. J. Hu, J. Y. Cao, Y. J. Huang, L. H. Xu, Y. Liang, D. Xiong, S. Guan, B. H. Guo, H. Q. Mai, Q. Y. Chen, X. Zhang, M. Z. Li, J. Y. Shao, C. N. Qian, Y. F. Xia, L. B.

Song, Y. X. Zeng & M. S. Zeng (2010). Epstein-Barr virus-encoded LMP2A induces an epithelial-mesenchymal transition and increases the number of side population stem-like cancer cells in nasopharyngeal carcinoma, *PLoS Pathog,* Vol.6, No.6, pp.e1000940, ISSN 1553-7374

A. Shinozaki, T. Sakatani, T. Ushiku, R. Hino, M. Isogai, S. Ishikawa, H. Uozaki, K. Takada & M. Fukayama (2010). Downregulation of microRNA-200 in EBV-associated gastric carcinoma, *Cancer Res,* Vol.70, No.11, pp.4719-27, ISSN 1538-7445

A. P. Malizia, N. Lacey, D. Walls, J. J. Egan & P. P. Doran (2009). CUX1/Wnt signaling regulates epithelial mesenchymal transition in EBV infected epithelial cells, *Exp Cell Res,* Vol.315, No.11, pp.1819-31, ISSN 1090-2422

M. D. Sides, R. C. Klingsberg, B. Shan, K. A. Gordon, H. T. Nguyen, Z. Lin, T. Takahashi, E. K. Flemington & J. A. Lasky (2011). The Epstein-Barr virus latent membrane protein 1 and transforming growth factor--beta1 synergistically induce epithelial--mesenchymal transition in lung epithelial cells, *Am J Respir Cell Mol Biol,* Vol.44, No.6, pp.852-62, ISSN 1535-4989

S. Z. Yang, L. D. Zhang, Y. Zhang, Y. Xiong, Y. J. Zhang, H. L. Li, X. W. Li & J. H. Dong (2009). HBx protein induces EMT through c-Src activation in SMMC-7721 hepatoma cell line, *Biochem Biophys Res Commun,* Vol.382, No.3, pp.555-60, ISSN 1090-2104

N. Delhem, A. Sabile, R. Gajardo, P. Podevin, A. Abadie, M. A. Blaton, D. Kremsdorf, L. Beretta & C. Brechot (2001). Activation of the interferon-inducible protein kinase PKR by hepatocellular carcinoma derived-hepatitis C virus core protein, *Oncogene,* Vol.20, No.41, pp.5836-45, ISSN 0950-9232

M. M. Lai & C. F. Ware (2000). Hepatitis C virus core protein: possible roles in viral pathogenesis, *Curr Top Microbiol Immunol,* Vol.242, pp.117-34, ISSN 0070-217X

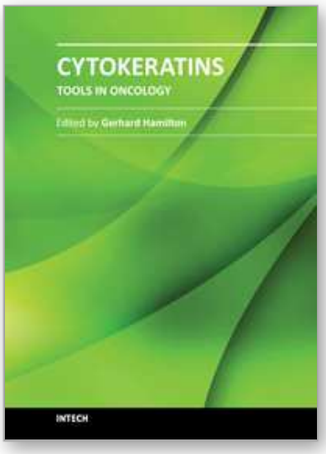
N. Zhu, A. Khoshnan, R. Schneider, M. Matsumoto, G. Dennert, C. Ware & M. M. Lai (1998). Hepatitis C virus core protein binds to the cytoplasmic domain of tumor necrosis factor (TNF) receptor 1 and enhances TNF-induced apoptosis, *J Virol,* Vol.72, No.5, pp.3691-7, ISSN 0022-538X

N. Pavio, S. Battaglia, D. Boucreux, B. Arnulf, R. Sobesky, O. Hermine & C. Brechot (2005). Hepatitis C virus core variants isolated from liver tumor but not from adjacent non-tumor tissue interact with Smad3 and inhibit the TGF-beta pathway, *Oncogene,* Vol.24, No.40, pp.6119-32, ISSN 0950-9232

S. Battaglia, N. Benzoubir, S. Nobilet, P. Charneau, D. Samuel, A. L. Zignego, A. Atfi, C. Brechot & M. F. Bourgeade (2009). Liver cancer-derived hepatitis C virus core proteins shift TGF-beta responses from tumor suppression to epithelial-mesenchymal transition, *PLoS One,* Vol.4, No.2, pp.e4355, ISSN 1932-6203

T. Li, D. Li, L. Cheng, H. Wu, Z. Gao, Z. Liu, W. Jiang, Y. H. Gao, F. Tian, L. Zhao & S. Wang (2010). Epithelial-mesenchymal transition induced by hepatitis C virus core protein in cholangiocarcinoma, *Ann Surg Oncol,* Vol.17, No.7, pp.1937-44, ISSN 1534-4681

P. M. Hofman (2010). Pathobiology of the neutrophil-intestinal epithelial cell interaction: role in carcinogenesis, *World J Gastroenterol,* Vol.16, No.46, pp.5790-800, ISSN 1007-9327



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The first chapters of the volume "Cytokeratins - Tools in Oncology" discuss multiple functions of cytokeratins in organization of the intermediary filaments in normal intestine and liver as well as microfold L cells and the usability of cytokeratins 7, 8 and 20 in tumor diagnosis in detail. Epithelial to mesenchymal transition as a mechanism important in pathogenesis is touched in another chapter, followed by several articles dealing with the role of cytokeratins for detection of disseminated tumor cells and as response markers during chemotherapy. This book is therefore destined to all cancer researchers and therapists who want to understand the diagnostic application of cytokeratins in histology and, especially, the use of anti-cytokeratin antibodies to identify viable residual tumor cells accounting for a higher risk of tumor recurrence or cancer cells responding to chemotherapy, respectively.

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