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REVIEW ARTICLES

Molecular mechanisms controlling the phenotype and the EMT/MET dynamics of hepatocyte

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Abstract

The complex spatial and paracrine relationships between the various liver histotypes are essential for proper functioning of the hepatic parenchymal cells. Only within a correct tissue organization, in fact, they stably maintain their identity and differentiated phenotype. The loss of histotype identity, which invariably occurs in the primary hepatocytes in culture, or *in vivo* in particular pathological conditions (fibrosis and tumours), is mainly because of the phenomenon of epithelial-to-mesenchymal transition (EMT). The EMT process, that occurs in the many epithelial cells, appears to be driven by a number of general, non-tissue-specific, master transcriptional regulators. The reverse process, the mesenchymal-to-epithelial transition (MET), as yet much less characterized at a molecular level, restores specific epithelial identities, and thus must include tissue-specific master elements. In this review, we will summarize the so far unveiled events of EMT/MET occurring in liver cells. In particular, we will focus on hepatocyte and describe the pivotal role in the control of EMT/MET dynamics exerted by a tissue-specific molecular mini-circuitry. Recent evidence, indeed, highlighted as two transcriptional factors, the master gene of EMT Snail, and the master gene of hepatocyte differentiation HNF4α, exhorting a direct reciprocal repression, act as pivotal elements in determining opposite cellular outcomes. The different balances between these two master regulators, further integrated by specific microRNAs, in fact, were found responsible for the EMT/METs dynamics as well as for the preservation of both hepatocyte and stem/precursor cells identity and differentiation. Overall, these findings impact the maintenance of stem cells and differentiated cells both in *in vivo* EMT/MET physio-pathological processes as well as in culture.

The liver is one of the most complex organs, from both a structural and functional standpoint. The physical interactions between different cell types, arranged in specific spatial relationships, are essential for proper functioning of the parenchyma. This appears evident in primary cultures, where the three-dimensional structure of the organ and the interactions with the other hepatic cell types are lost. Preserving liver cell differentiated phenotype *in vitro*, thus, still represents a challenge which is far from being overcome.

Hepatocytes, the major liver parenchymal cell type, appear to be pivotal, together with the vascular tree, for tissue organization and correct cross-talk between different cell types. The mature hepatocytes, with their arrangement in the liver lobule and paracrine activity, are in fact essential for a correctly organized sinusoidal system and control of the function of biliary cells and hepatic stellate cells (HSCs). Endothelial cells, cholangiocytes and stellate cells, in turn, are essential for maintaining correct functionality of the hepatocyte. The importance of tissue architecture for correct functioning of the liver is further demonstrated by the phenomenon of liver zonation, in which hepatocytes display a metabolic specialization depending on their localization in the liver lobule. The zonation is related to large modulations of gene expression induced by diffusible factors present in the liver lobule according to concentration gradients along the portal-venular axis (1). Recently, the Wnt pathway has been unveiled as the main molecular signalling of the liver zonation, and cross-talk between its downstream effectors and liver-specific transcriptional factors has been described (2).

In the adult liver, the hepatocyte stable identity is challenged when the cells must undergo changes in response to intrinsic and extrinsic signals, such as those

inducing and driving the response to mitogens during compensatory liver regeneration.

After loss of parenchyma (for surgical resection or injuries caused by drugs, toxins or acute viral diseases), virtually all residual hepatocytes, that in the normal organ are nearly all quiescent, undergo 2–3 cell replications, completely recovering the original liver mass. This liver compensatory regeneration is a rapid and tightly orchestrated phenomenon, efficiently ensuring the reacquisition of original tissue functionality (3). Most importantly, and differently from other cell types, in which the proliferation and terminal differentiation are mutually exclusive activities, in liver regeneration the mature hepatocytes re-enter in cell cycle and proliferate while maintaining the liver vital functions(4, 5). If starting from this evidence it has been concluded that, essentially, proliferating hepatocytes do not lose their differentiated phenotype, a number of observations also suggest as proliferation might imply the modulation of some features towards a prenatal developmental stages; in regenerating liver in fact: (i) the junctional complexes of hepatocytes resemble the embryonic stages (6), (ii) the transcriptional factor C/EBPα (known to peak around birth, to decrease in the immediate neonatal period and to rebound in the adult (7) is down-regulated (8), (iii) subpopulations of hepatocytic cells expressing Fn-14 and other progenitor markers are detectable (9) and as recently reported, (iv) E-cadherin is down-regulated (10). This appears consistent with results obtained in *in vitro* models, where comparative proteomic analysis indicates as the hepatocyte terminal differentiation programme requires a quiescent state maintained by cell–cell contact through the E-cadherin/β-catenin pathway (11).

Liver cells undergo cellular transitions

The idea that terminal differentiation is permanently maintained, once development is complete, has been challenged by the observations that hepatocytes are highly responsive to stimuli inducing profound cell reprogramming and resulting in a mesenchymal transdifferentiation by the process known as epithelial-to-mesenchymal transition (EMT).

The EMT is a complex phenomenon by which several types of epithelial polarized cells lose cell–cell connections and acquire mesenchymal characteristics of motility and invasiveness (12). Different EMT subtypes can be categorized according to when this process occurs: (i) type 1 concerns developmental and organogenesis events; (ii) type 2 plays a major role in wound healing, regeneration and fibrosis and (iii) type 3 characterizes epithelial tumour progression rendering cancer cells able to metastasize [for review (13)].

Hallmarks for EMT include increased expression of vimentin, nuclear localization of β-catenin and production of transcription factors able to inhibit E-cadherin expression. In particular, master EMT inducers have been identified in the transcriptional repressors belonging to the Snail family, Snail (Snai1) and Slug (Snai2); these factors are able to determine EMT induction targeting many epithelial genes starting from the direct inhibition of E-cadherin gene transcription(14). The first indication that the Snail gene family had a key role in triggering EMT, by loss-of-function experiments in chick embryos (15), was successively confirmed in several epithelial cell types and other vertebrate embryos (16, 14). It is now widely demonstrated that Snail levels are inversely correlated with E-cadherin in numerous tumours and contribute to the acquisition of an invasive phenotype (14, 16–19).

Many signalling pathways, including transforming growth factor β (TGFβ) superfamily, epidermal growth factor (EGF), Wnt, sonic hedgehog (Hh) and Notch, and oncogenic events, such as Src or Ras activation, are implicated in EMT induction, both in physiology and pathology (20, 21). Notably, Snail is the main effector of many of these EMT inducers (22).

In particular, TGFβ1 is considered the master EMT inducer for malignant and non-malignant epithelial cells, including hepatocytes (23). TGFβ1 acts as a potent inducer of EMT combining both Smad-dependent and independent signalling pathways (24, 25). Binding of TGFβ1 with its receptors type I and type II induces Smad2/3 phosphorylation. Phosphorylated Smads recruit Smad4 to translocate into the nucleus and regulate gene expression. The activation of Snail promoter depends on the direct binding of Smad3/Smad4 (26). In turn, a complex between Snail and Smad3/Smad4 represses the epithelial markers E-cadherin and occludin (26). TGFβ also promotes EMT through activation of MAPK, Rho GTPases and PI3K (24). Other EMT transcriptional regulators, such as ZEB1, are also regulated by TGFβ1 in a Smad-dependent manner (24, 25). Notably, Snail (27) and ZEB1 (28) are able to repress miR-200 family members, in turn targeting ZEB1 (29), and thus controlling the EMT outcome by a feedback mechanism. Several other microRNAs and some ncRNAs, with their ability to target multiple components involved in epithelial integrity or mesenchymal traits, recently emerged as potent regulators of EMT/MET (30–32).

The EMT reverse transdifferentiation event, the mesenchymal-to-epithelial transition (MET), allows the mesenchymal cells to redifferentiate into epithelial structures. MET occurs in physiological (i.e. in development) and pathological situations (i.e. cancer metastasis), where the migrating mesenchymal-like cells that have reached secondary sites reacquire cell–cell contacts and polarity (33). Notably, an event of MET resulted necessary for the success of experimentally induced cellular reprogramming, where a cocktail of critical transcriptional factors prompts fibroblasts to mimic epithelial-like undifferentiated Embryonic Stem Cells (ESC) (34, 35). Coherently with the fact that MET represents a reversion of EMT, a down-regulation of

EMT-inducing transcription factors such as Snail, Slug, and ZEB1 is invariably associated with MET. As yet MET molecular mechanisms are only partially characterized.

It is conceivable that the MET process may involve a number of histotype-specific elements, as suggested by evidence revealing as the BMPs-dependent signalling can promote MET in a histotype and context-specific manner (24, 35).

The EMT/MET dynamics has also been proposed as a key element of stem cell biology (36). In fact, a common characteristic of embryonic and adult stem cells is the presence of both epithelial and mesenchymal traits that define the 'metastable' stem/precursor phenotype. A fine regulated balance of EMT/MET dynamic allows for stem cell self-renewal while its unbalance in either direction causes generation of precursors that differentiate into epithelial or mesenchymal cell types. The molecular pathways controlling the EMT/MET dynamics of normal stem cells are expected to be similar to that of cancer stem cells, a specific population of metastable tumour cells able to initiate and maintain many types of cancer (37–39). Of note is the theoretical model for molecular mechanisms controlling EMT/MET dynamics based on the miR-34/Snail and the miR-200/Zeb mutual-inhibition feedback circuits (40).

In the liver, several types of cells have been shown to undergo EMT/MET: in particular, hepatocytes, both primary and established in line, when treated with TGFβ down-regulate epithelial and hepatic markers (e-cadherin and albumin), while up-regulate mesenchymal genes (vimentin and alpha-SMA) and acquire motility and invasiveness (41–44). Valdes and colleagues (45) reported that TGFβ treatment of primary hepatocytes selects an apoptosis-resistant cell population, which is subjected to an EMT with an increase in Snail and vimentin and negative regulation of E-cadherin, cytokeratin 18 and hepatocyte nuclear factors (HNFs). For most of these TGFβ effects, the activation of Focal Adhesion Kinase (FAK) signalling is required. TGFβ, in fact, induces a Src-dependent activation of FAK which has been demonstrated to be necessary for (i) transcriptional up-regulation of mesenchymal and invasiveness markers and (ii) delocalization of membrane-bound E-cadherin (46).

Moreover, the EMT process has been related to the dedifferentiation process that invariably occurs in freshly isolated hepatocytes within a few days of culture on a stiff layer of dried collagen. Recently, the mechanism controlling dedifferentiation of hepatocytes in primary cultures, in relation to the specific signalling network triggered by extracellular matrix, has been partially unveiled. In this case, a FAK-mediated AKT activation (promoting a resistance to the TGFβ-induced apoptosis) and ERK1/2 signalling activation (opening the route to a TGFβ-induced EMT programme) were also observed. Antagonizing Akt and ERK1/2 signalling pathways caused the rescue of functional and morphological features, thus unveiling a mechanism controlling hepatocyte differentiation (47).

Cholangiocytes also transdifferentiate in mesenchymal-like cells when treated with TGFβ (48) or cultured in medium conditioned by myofibroblasts derived from fibrotic livers (49). Regarding HSCs, the liver cell type that most contributes to liver fibrosis, they have been suggested to be transitional cells. As a matter of fact HSC retain precursor features in expressing a number of epithelial and mesenchymal markers and appear able in culture to become myofibroblasts by EMT or hepatocytes with a MET-like process (50).

With respect to the relevance of the EMT/MET phenomena occurring in *in vivo* adult liver, the literature collected evidence both for and against it. Concerning the HSCs, if the idea that these are transitional cells, expressing both mesenchymal and epithelial genes, is accepted (50, 51), their activation into myofibroblasts, largely observed during liver fibrosis, corresponds to an EMT (51, 52). Instead, the possibility of HSCs undergoing MET *in vivo* is most debatable. An elegant work by Yang and collaborators (53), taking advantage of a genetic cell lineage tracing, showed that HSCs, when activated by liver injury or culture, became highly proliferative and started to express mesenchymal and epithelial markers. From these transitional cells, a population of genetically marked hepatocytes, able to repopulate large areas of the hepatic parenchyma, emerged. Consistent with these results is the recent evidence, collected by Conigliaro *et al.* (54), that metastable hepatic stem/progenitor cells, isolated from embryonic livers and established in clonally derived cell lines, give rise to both hepatocytes and HSCs when injected into normal growing liver.

Regarding hepatocytes, the results gathered by Zeisberg *et al.* (55), based on a lineage tracing approach in a murine experimental model of liver fibrosis, suggested that collagen producing cells can also be derived from hepatocytes by EMT and that these types of myofibroblasts contribute significantly to the progression of liver disease. However, Taura *et al.* (56) have followed, by a similar approach of cell fate mapping, the outcome of albumin expressing cells in injured liver, without identifying hepatocyte-derived fibroblasts.

The Fig. 1 summarizes in a schematic representation the physio-pathological EMT/MET dynamics of liver cells.

EMT in hepatocytes implies down-regulation of hepatic master gene HNF4α

The EMT in hepatocytes correlates with the down-regulation of the hepatic differentiation key factors HNFs (hepatocyte nuclear factors), particularly HNF4α.

Cicchini and colleagues demonstrated, indeed, that the TGFβ treatment of well-differentiated, non-tumourigenic, hepatocytic cell lines (57) induces a full EMT, underlined by Snail induction and E-cadherin, HNF1

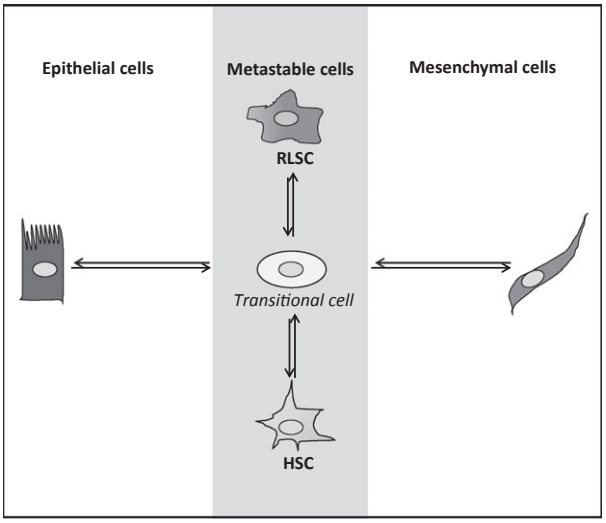

Fig. 1. Schematic representation of physio-pathological EMT/MET dynamics of liver cells. Cells coexpressing epithelial/mesenchymal markers are here termed 'metastable'.

and HNF4α (43) down-regulation. Snail appears to be at the cross-road of epithelial morphogenesis and differentiation of hepatocyte. Snail overexpression, in fact, appeared sufficient (i) to induce EMT in hepatocytes (with conversion of morphology, down-regulation of several epithelial adhesion molecules, reduction of proliferation and induction of matrix metalloproteinase two expression) and, most relevantly, (ii) to directly repress the transcription of the HNF4α gene (43).

The liver-enriched transcriptional factor (LEFT) HNF4α is a well-known master of both liver morphogenesis and hepatocyte differentiation. During embryonic development, a highly regulated network among a number of LEFTs drives the differentiation process of the endodermal stem cell in differentiated parenchymal cells (58): HNF4α1, HNF1β, FoxA2, HNF6 and LRH-1 are key factors in this circuitry by acting as positive regulators of each other's and of a repertoire of hepatic target genes. In particular, HNF1β, FoxA2 and HNF6 have roles in controlling the onset of the hepatic gene expression during specification of the liver progenitors, while HNF4α, although not having an impact on hepatic specification, is essential for subsequent differentiation of hepatic progenitors. During midgestation, HNF4α drives hepatocyte differentiation controlling the acquisition of an epithelial phenotype (59). This implies the activation of the expression of cell adhesion and junction molecules that in turn convert non-polarized cells in organized sheets of closely associated polarized epithelial cells.

Concerning liver morphogenesis, when HNF4α is specifically removed from foetal hepatoblasts, hepatic architecture is severely affected, with livers exhibiting loss of endothelial cells and disrupted hepatocellular polarity (60). In adult hepatocytes, HNF4α continues to have an important role in maintaining hepatocyte functions (61, 62) and proliferative quiescence (63). This is confirmed by genome-wide ChIP studies in which HNF4α was found to occupy 12% of genes in human adult hepatocytes (64).

Notably, an inverse correlation between HNF4α and the EMT master factor Snail was demonstrated during hepatocyte differentiation (65) and hepatocellular carcinoma (HCC) progression. Snail expression, in fact, significantly increases along with HCC dedifferentiation, accelerating cancer invasion (66). Moreover, EMT and E-cadherin down-regulation have been shown to play an important role in HCC progression (67). On the other hand, the loss of HNF4α expression is determinant for HCC progression and its forced re-expression is able to promote the highly invasive undifferentiated tumour reversion towards a more differentiated epithelium and the reacquisition of cell-ECM contacts (68). Moreover, HCC cells expressing HNF4α also re-established expression of the profile of liver transcription factors and hepatic genes that are associated with a differentiated hepatocyte phenotype. Notably, HNF4α overexpressing hepatoma cells are deficient for Snail, expressed instead in the parental population (69).

All this evidence suggests that Snail could contribute to liver tumour progression through the down-regulation of HNF4α. This is in agreement with other observations that dedifferentiated hepatoma cell lines constitutively express Snail, while well-differentiated rat hepatoma cells are deficient for Snail (43).

HNF4α acts as a met master factor

Various evidences demonstrated a role for HNF4α as a dominant regulator of the epithelial phenotype. Indeed, HNF4α not only regulates the developmental expression of adhesion molecules (60) but also when ectopically expressed in different cells, such as fibroblast (59) and F9 cells (70), it is sufficient to trigger tight-junction and epithelial polarity molecules expression inducing a MET.

Interestingly, HNF4α expression in dedifferentiated hepatoma is sufficient to re-establish the hepatocyte markers gene expression and epithelial cell morphology and polarity (69). Moreover, in HCC the forced expression of HNF4α induces reversion of highly invasive tumours towards a less invasive phenotype (68).

Notably, enforced expression of HNF4α, plus Foxa1, Foxa2 or Foxa3, directly reprograms mouse fibroblasts into induced hepatocyte-like (iHep) cells (71).

HNF4α controls hepatocyte epitheliality by active repression of the mesenchymal programme

As mentioned above, the main HNF4α characterized function was the positive modulation of tissue-specific epithelial and hepatocyte gene expression, sufficient for the acquisition of an epithelial differentiated phenotype.

Moreover, a new mechanism was recently unveiled by which HNF4α, inducing MET maintains the hepatocyte-differentiated phenotype. Santangelo and colleagues (72), indeed, demonstrated a novel HNF4α 'anti-mesenchymal' role through the orchestrated repression of both master EMT regulators and mesenchymal genes. These authors provided evidence of repression of the mesenchymal programme, both during the HNF4α-mediated MET process of undifferentiated hepatoma or fibroblast cells, and in the normal fully differentiated hepatocytes that stably retain the epithelial phenotype. In particular, in dedifferentiated hepatoma, forced expression of HNF4α appeared sufficient to down-regulate mesenchymal markers such as Snail, Slug, HMGA2, vimentin and fibronectin expression. In terminally differentiated hepatocytes, endogenous HNF4α was found stably recruited to the promoters of these EMT inducers and mesenchymal genes. Using both cell cultures and liver-specific HNF4α knockout mouse models, a direct correlation between loss of HNF4α and up-regulation of the mesenchymal genes has been demonstrated. Histological examination of liver sections from Alb-HNF4α -/- mice demonstrated that hepatocytes, with the known hypertrophic phenotype, express vimentin, desmin, fibronectin and α-smooth muscle actin (with no increase in non-parenchymal cells) (61,72).

These data integrate the well-established notion of the pivotal positive role of HNF4α in hepatocyte differentiation through expression of epithelial genes with the new concept of an active and fundamental role of HNF4α in the repression of the hepatocyte mesenchymal programme.

The capacity of HNF4α-depleted hepatocyte to acquire a mesenchymal phenotype is intriguing and deserves future investigations on HNF4α KO models, especially in the light of the reports suggesting that quiescent HSC might be transitional cells derived from the partial EMT of epithelial cells (73). The evidence reported by Santangelo and colleagues (72), together with the previous demonstration that Snail directly represses HNF4α (43), allows for the formulation of a simple cross-regulatory model between Snail and HNF4α: the expression of each factor is mutually exclusive to the other, and this is because of the presence of repressor elements in each promoter. This HNF4α/Snail circuit of reciprocal regulation between two master regulators provides a simple molecular mechanism for EMT/MET feedback and reversible differentiation processes in both physiology and pathology.

Notably, the same authors extended the 'anti-mesenchymal' role of HNF4α, by direct transcriptional regulation of EMT master genes and mesenchymal markers, the other hepatocyte-enriched transcriptional factor HNF1α (72), known to play an important role in hepatocyte differentiation and function (64) and to be positively regulated by HNF4α. Successively, also Pelletier and colleagues, providing evidence that HNF1α inhibition triggers EMT in human liver cell lines, concluded that HNF1α has a role in the maintenance of hepatocyte epithelial identity (74). Moreover, was reported to be one of the few factors which ectopic expression in embryonic fibroblasts (MEFs) appears sufficient to generate induced hepatocyte-like (iHep) cells (75).

Thus, both HNF4α and HNF1α transcriptional repression of critical mesenchymal genes is pivotal, not only for the regulation of the dynamic process of MET but also for the maintenance of a stable epithelial phenotype.

SNAIL/HNF4α circuit also plays a pivotal role in liver stem cell maintenance

Several recent studies showed how the induction of EMT in differentiated epithelial cells corresponds to the execution of a stemness programme. In particular, an important work of Mani and collaborators showed that treatment with TGFβ of immortalized cells of mammary epithelium, as well as ectopic expression of two master genes of the EMT, Twist or Snail, induces, together with a mesenchymal phenotype, the expression of stem cell markers and the ability to form mammospheres, as mammary epithelial stem cells do (76). Similarly, the treatment of tumoural mammary epithelial cells, allows for the acquisition of a cancer stem cell behaviour, such as the formation of mammospheres, the capacity to form colonies on soft-agar and increased efficiency of tumour formation. Also Morel *et al.* (77), using a mammary tumour progression model, showed that the acquisition of stem and tumourigenic characteristics of cancer stem cells are driven by EMT induction.

Moreover, the coexpression of epithelial and mesenchymal markers in the epithelial stem cells characterized so far, suggests that the maintenance of a partial mesenchymal programme could be necessary for stemness behaviour.

In line with this observation is the finding that Snail is expressed in hepatic stem/precursor cells resident in the murine livers and that this expression correlates with the expression of stemness markers (27). This unexpected finding, considering that the transcriptional repression is the only function so far attributed to Snail, allowed for identifying other factors integrating/mediating Snail activity. In particular, it has been shown that in liver stem/precursor cells, Snail inhibits the hepatospecific programme through direct repression of the HNF4α gene and the epithelial microRNAs (miR)-200c and 34a, known as stemness-inhibiting microRNAs for their targeting of stemness genes, while in the hepatocytes, HNF4α, together with a direct repression of Snail gene, directly up-regulates miR-200 family members (200a, b and c) and miR-34a transcription, thus further stabilizing the hepatocytic phenotype. Altogether, these data unveiled Snail, HNF4α and miRNAs as elements controlling hepatic stem cell maintenance/differentiation, acting in a simple and direct molecular mini-cir-

cuitry in which master elements reciprocally repress their own expression while inversely controlling the expression of specific microRNAs.

HNF4α as a gene therapy tool for HCC

As mentioned above, the emergence of mesenchymal traits characterizes cells metastasizing from carcinomas and during HCC progression, an inverse regulation between Snail and HNF4α is observed with Snail expression directly correlating with the dedifferentiation grade of HCC, cancer invasion and poor prognosis (66). In a comparable way, HNF4α expression, lost in more aggressive HCC, if restored, promotes reversion towards a less invasive phenotype, both repressing EMT programme and promoting hepatocyte differentiation (69, 78). Therefore, the restoration of the functions HNF4α in HCC invasive represents an important milestone for the anticancer therapies. However, recent data have shown that in an environment containing TGFβ, HNF4α restoration usually fails to counteract tumour progression. The tumour promotion effects of TGFβ, indeed, result dominant on HNF4α activity, thus limiting the HNF4α gene transfer as a therapeutic approach of HCC.

The molecular basis of this dominance resides on some post-translational modifications, induced by the TGFβ signalling on HNF4α protein, that provoke the displacement of transcriptional factor from its target gene promoters, including Snail. GSK3β kinase has been individuated as one of the TGFβ targets mediating HNF4α functional inactivation; GSK3β chemical inhibition, in fact, resulted in the HNF4α DNA binding impairment, while a constitutively active GSK3β mutant impairs the TGFβ-induced inhibitory effect on HNF4α tumour suppressor activity (79).

Conclusions and perspectives

The balance between Snail (EMT master gene) and HNF4α (MET master gene) in liver stem cells and liver cancer cells (cancer stem cells or transformed hepatocytes) ultimately influences the outcome of the transition between the mesenchymal/undifferentiated and the epithelial/differentiated phenotype. This simple and direct molecular mini-circuitry of master elements (Fig. 2), able to reciprocally repress their own expression may theoretically provide the best device to trigger complex phenomena such as stemness and differentiation. However, this necessarily implies that these master factors act in much more complex macromolecular systems, able to direct and modulate a whole transcriptional profile, influencing the early stages of gene expression.

Further studies are necessary to shed light on the complexity of the cellular output of the proposed mini-circuitry, based on the conceivable hypothesis that a transcriptional master gene acts by organizing a molecular platform that in turn is capable of driving a coherent cellular response which involves hundreds of target genes. In other terms, both the EMT/stemness master regulator Snail and the MET/hepatocyte differentiation master regulator HNF4α could behave as reprogramming transcriptional factors, triggering the necessary epigenetic changes on regulative regions of their own target genes. As 'master' regulators of stem cell fate and/or the reprogramming of a differentiated cell, they could directly recruit general chromatin modifiers to their target genes, regulating their transcriptional competency.

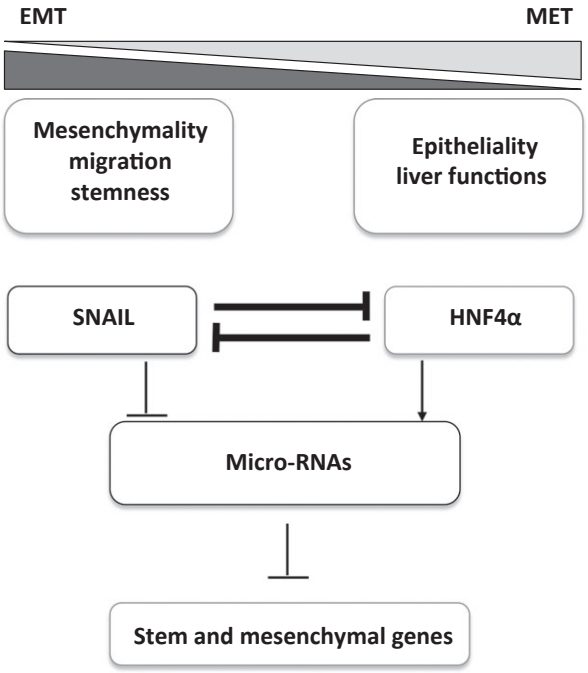

Fig. 2. Molecular circuitry controlling EMT/MET liver cell dynamics. The balance between Snail and HNF4α, based on their mutual negative regulation, controls epitheliality, mesenchymality and stemness of liver cells. A number of microRNAs were found to amplify the biological impacts of the circuitry.

Moreover, we believe that the hierarchical relevance of these master regulatory molecules, controlling a broad range of cellular functions, may allow for designing simple molecular therapies based on a gene transfer approach. The epistatic relation among Snail/HNF4α, a number of miRNAs and their target genes, is further influenced by environmental cues such as TGFβ.

In this context, the use of engineered molecular tools, insensitive to negative regulation by the microenvironment, will represent the successful approach to improve gene therapy strategies.

Although promising in *in vitro* and preclinical studies, this strategy, when applied clinically, should take into account the tumour niche influence, which is capable of exerting direct functional impairment of therapeutic molecules.

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