



Phenotype: the result of a complex and still largely unknown interplay between molecules

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Cholangiocarcinoma (CCA) is a highly malignant tumor and the second-most common primary liver cancer. Chronic inflammation and cholestasis predispose to CCA. Previous work showed a role of c-MYC upregulation in cholestatic liver injury (1), and during CCA progression using a murine model of cholestasis-associated CCA (2). Furthermore, the Maf family proteins, among which MafG and c-Maf, were found to contribute to cholestatic liver injury induced in mice by bile duct ligation (BDL) (3). The overexpression of MafG and c-Maf has been shown to be associated, in mice, with lower GSH level during BDL (3).

S-adenosylmethionine (SAM), the major methyl donor of mammalian cells, favors reactions catalyzed by methyltransferase enzymes (4), and donates its methyl groups to a large number of molecules, including nucleic acids, proteins, carbohydrates, and lipids.

SAM is mainly synthesized in normal liver by methionine-adenosyltransferase1A (MAT1A), a marker of highly differentiated hepatocytes (5).

MAT1A enzymatic activity decreases in patients with chronic liver disease and in MAT1A-KO mice. MAT1A-KO mice, characterized by chronic SAM deficiency, spontaneously develop liver steatosis and hepatocellular carcinoma (HCC) (5,6). MAT1A activity decreases also in HCC chemically induced in rodents and in human HCC (6-8). Reactivation of MAT1A reduces liver tumor growth and metastasis (9).

c-MYC plays an important role during both liver injury and HCC progression; it has a broad effect in a plethora of oncogenic processes. c-MYC overexpression has been detected in up to 70% of human cancers and is linked to tumor aggressivity (1). It is also overexpressed in preneoplastic and neoplastic experimental liver lesions (10)

and in human HCC (11).

Interestingly, SAM administration during the development of chemically-induced liver cancer in rats, reduces c-Myc expression and significantly decreases the progression of dysplastic liver nodules to HCC (12). These findings are in keeping with the recent observation that SAM level regulates c-Myc expression in liver and mouse hepatocytes. Low SAM level associated with MAT1A loss, as in *Mat1a*-KO mice, leads to a marked increase in c-Myc mRNA level (13). SAM decreases significantly during chronic cholestasis. In contrast SAM administration is protective against cholestatic liver injury, caused by BDL or lithocholic acid (14), and cholestasis of pregnant women (15).

Taking into account the complex and well-known role played by MAT1A and its product SAM, during liver injury and during HCC development and progression, Dr. Yang and coworkers, in a recent work (16), extended the study of MAT1A and SAM synthesis deregulation to chronic cholestasis and CCA. The Authors observed a significant decrease of MAT1A expression in epithelial bile duct cells and in hepatocytes of mice after two weeks of cholestasis, induced by BDL or by lithocholic acid. The decrease occurred both at mRNA and protein levels, suggesting a pre-translational mechanism. This was associated with a strong upregulation of c-Myc, c-Maf and MafG genes, whose expression is low in normal liver (17).

Interestingly, Yang and coworkers found that in normal liver Mat $\alpha 1$ protein interacts mainly with Mnt, Max and, at lower extent, with c-Myc, c-Maf and MafG proteins, affecting reciprocal interactions between these proteins. Mnt is a member of the Myc/Max/Mad network of

Table 1 Effects of the binding of MAT1A, c-Myc, c-Maf and MafG proteins on target genes promoter activity

Transfected gene	Promoter activity			
	<i>MAT1A</i>	<i>c-Myc</i>	<i>MafG</i>	<i>c-Maf</i>
<i>MAT1A</i>	↑310%	↓90%	↓30–40%	↓30–40%
<i>MAT1A mut</i>	↑80%	No effect	No effect	No effect
<i>c-Myc</i>	↓75%	↑350%	↑100%	↑800%
<i>MafG</i>	↓42%	–	–	–
<i>c-Maf</i>	↓35%	–	–	–
Co-transfection	–	–	–	–
<i>c-Myc/MAT1A</i>	–	–	No effect	No effect
Silencing (siRNA)				
<i>MAT1A</i>		↑200%		
<i>c-Myc</i>	↑150%	↓50%	↓20–30%	↓20–30%
<i>MafG</i>	↑60%	↓20%	↓50 %	↓10–20%
<i>c-Maf</i>	↑50%	↓40%		↓50%

transcription factors, which regulates cell proliferation, differentiation, cellular transformation and tumorigenesis. It is a Max-interacting transcriptional repressor antagonizing both the proliferative and proapoptotic functions of c-Myc *in vitro* (18). In chronic cholestasis, low Mat1A expression is associated with a decrease in the above interactions. In contrast, there is an increase of the c-Myc, c-Maf and MafG reciprocal interactions.

Noticeably, an E-box sequence is present in the promoter regions of *MAT1A*, *MNT*, and *c-MYC*, *c-MAF* and *MAFG* genes. The E-box motif, interacting with elements involved in genes expression, may strongly affect the dynamic interactions between these molecules, and may modulate differently the activity of their promoter. Thus, the binding of each of these regulatory molecules to the E-box of the promoters regions of *c-MYC/C-MAF/MAFG* respectively, modulates the expression of the target genes of each of these molecules. According to Yang and coworkers' results (16), the E-box serves as a repressor element for MAT1A targeting c-MYC, C-MAF and MAFG, while it positively regulates the MAT1A own expression.

Table 1 summarizes the complex and specific interplay, between MATA1, MNT, MAX, and c-MYC, c-MAF and MAFG proteins, affecting epithelial cells growth during chronic cholestasis and CCA, according to the transfection experiments, made by Yang and coworkers (16).

The interesting observations of Yang and coworkers (16) suggest the existence of an integrated functional relationship

between several molecules, contributing to chronic cholestasis and CCA pathogenesis. The availability of molecules and the ways by which they reciprocally interact each other, may modify deeply their functional behavior.

Therefore, the phenotypic manifestations of any single tumor, either HCC or CCA, would be the result of greatly complex interactions between genetic information and multiple post-transcriptional and post-translational modifications. The knowledge of this regulatory molecular network is essential in view of the widespread idea that each tumor needs a personalized therapy.

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of interest to declare.

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