Editorial

MAF proteins: a family of regulating and regulated molecules

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The proteins of MAF family (the cellular counterpart of viral oncogene MAF, isolated from avian musculoaponeurotic fibrosarcoma) are transcription factors regulating gene expression. On the bases of the size, they are sub-grouped into two families: the "large" (L-MAFs: MAFA, MAFB, c-MAF, and Nrl) and the "small" (S-MAFs: MAFF, MAFK, and MAFG) MAF proteins (1).

The MAFs are evolutionary conserved among vertebrates, and they are expressed in a wide range of tissues.

All MAFs harbor a basic sequence binding DNA, in turn linked to a leucine zipper domain (b-ZIP), and forming homo/hetero-dimers with transcription factors containing the b-ZIP-region. L-MAFs recognize a T-MARE region, containing TPA responsive elements (TRE), and a C-MARE region, containing cAMP responsive elements (CRE). MARE motifs are flanked by three conserved residues "TGC" and "GCA", at their 5'- and 3'-ends, respectively. These flanking nucleotides guarantee the inherent ability to recognize specific DNA sequences (2).

Regulation of MAF proteins expression and function

The transcriptional activation domain (TAD) drives the effect of l-MAF subgroup. S-MAF, lacking TAD sequence, exert a positive or a negative regulatory activity, depending on specific partner and cellular context. S-MAF homodimers repress gene transcription, by binding to MAF recognition elements MARE (TGCTGACTCAGCA), within the target genes (1).

In addition to the transcription regulators containing bZip regions, Jun, Fos, and Bach1, and several other factors heterodimerize with v-/c-MAF proteins. The component of the helix-loop-helix zipper transcription factors, USF2 inhibits c-MAF. In turn, c-MAF forms with c-Myb a transcriptionally inert complex, involved to guide the development of myeloid cells lineage. Similarly, MAFB represses the transcriptional activity of c-Ets-1, inhibiting erythroid cells differentiation (3).

Many observations reveal the importance of s-MAFs in various biological pathways, and underline that a variety of signals modulates s-MAF functions, at transcriptional and/or post-transcriptional levels.

S-MAFs heterodimerize with NF-E2-related factor 2 (Nrf2), Nrf1, or Nrf3, as with Bach1 and Bach2 factors, functioning as transcriptional activators or repressors.

S-MAFs positively modulate hypoxic response by binding the ARE (antioxidant responsive element) sequence of HIF-1α gene. The hypercapnia induces *MAFG*-mRNA in the central baroreceptive neurons. Phenylephrine induces MAFG activation of the baroreceptors. MAFG and its variant MAFG-2 expression decrease, when the extracellular environment shows an imbalance of its acid/base composition, which favors MAFG dimerization with FosB, and the DNA binding activity of the heterodimer (4). The wild-type protein, but not the sumoylation defective MAFG mutant, represses target gene expression *in vivo*, suggesting the critical role of sumoylation for MAFG activity. Novel studies revealed that only MAFG conjugated to SUMO2/3, exerts repression activity. In addition, sumoylation at Lysine 14 is required for s-MAFs homodimer mediated repression (5).

Three alternative non-coding first exons drive s-MAF

expression, in response to specific stimuli. Two different promoters of *MAFK* gene mutually mediate the specific expression in mesodermal, and brain tissues (5).

The heterodimer MAFG/NF-E2p45 related factor 2 (Nrf2) interacts with CBP, when this protein binds CREB, the transcription cofactor cAMP-response element-binding protein. The acetylation of the MAFG/NF-E2p45 heterodimer is required and increases DNA-binding activity (6).

MAFs act as differentiation factors for specific cell types. S-MAFs must occupy the regulatory region of globin gene to induce erythroid differentiation (6). L-MAF and AP-1 bZIP transcription factors (activator protein-1 bZIP) regulate T cell function. The expression of interleukin-4 gene and the differentiation of Th2 cells, the subset of T-lymphocytes, is strictly regulated by C-MAF (7). The MAFK over-expression represses MARE-dependent transcription of T cells, lowering IL-2 and IL-4 cytokines secretion, and thymocyte cells proliferation (8).

L-MAF activates lens crystalline gene through MARE sequences (9). Brown adipose tissue, liver, heart and lungs, all show an increase of MAFG expression when ground squirrels undergo to hibernation; this event is followed by higher nuclear accumulation of MAFG and Nrf2. On the bases of these observations, an important role seems to be played by MAFG in the induction of heme-oxygenase during mammalian hibernation (9).

MAFG gene is expressed in liver, the major metabolic site for bile acid synthesis from cholesterol. Bile acids, potent signal transducer molecule, modulate metabolic pathways affecting bile homeostasis, lipid, and glucose metabolism. The nuclear farnesoid X receptor (FXR) controls the expression of cholesterol hydroxylases Cyp7a1 and Cyp8b1, the two key enzymes involved in bile acid synthesis (10). FXR downregulates bile acids synthesis, and increases their clearance. A dysfunction of FXR leads to dysregulated bile acid metabolism, abnormal lipoproteins and glucose metabolism. Activated FXR binds to promoter regions of target genes, as SHP (short heterodimer partner), or fibroblast growth factor 19 (FGF 19), and several transporters, bile salts export pump, and organic-solute-transporter α/β. SHP suppresses CYP7A1 in the liver (10). MAFG activated by FXR, represses bile acids synthesis. FXR activation favors an anti-inflammatory and anti-cholestatic environment, reducing liver exposure to toxic bile acids. Interestingly, MAFG overexpression modulates the composition of bile acid pool, but not the final pool size. MAFG represses cholic acid, but it increases muricholic acid levels.

Diseases and MAF expression

Several diseases are associated to s-MAFs loss-of-function, as progressive neuronal degeneration, cataract, thrombocytopenia cardiovascular disease, and embryonic lethality. S-MAFs/Nrf2 complex is involved in diseases prevention (4). Impaired S-MAF/Nrf2 decreases antioxidant and xenobiotic metabolism, it increases susceptibility to exogenous chemicals (e.g., acetaminophen) and to certain endogenous toxins (e.g., electrophilic compounds and reactive oxygen species), and prolongs inflammation, favoring various neurodegenerative disorders and arteriosclerosis (11). Nrf2, interacting with the KEAP1 protein, is blocked in the cytoplasm under basal conditions, thereafter it is ubiquitinated and degraded. Although loss-of-function mutations of *KEAP1*, and/or oncogenic signaling pathways contribute to the constitutive activation of Nrf2 in tumors, the heterodimer s-MAF/Nrf2 may sustain Nrf2 activation, tumor growth and progression (12).

Hypoxia activation of HIF-1 requires a series of events, involving stabilization of HIF-1α subunit, its phosphorylation, nuclear translocation, and interaction with several cofactors, among which the presence of the HIF-1α/MAFG complex in cells nuclei is essential (4). Hep3B cells show HIF-1α nuclear accumulation, while in MAFG knockdown, HIF-1α remains in the cytoplasm, suggesting that MAFG favors HIF-1α detection in the nucleus during ypoxia (4).

Small MAFs play a fundamental role in the pathology of diabetes. MAFA favors insulin gene transcription and pancreatic beta cell function. Impaired insulin availability and defective pancreatic function, induced in mice lacking MAFA ($MAFA^{-/-}$), is associated to development of diabetes mellitus (DM). Even though hyperglycemia develops in transgenic mice ($MAFK^{+}$), whose pancreatic beta cells overexpress MAFK, these animals do not develop DM. Taking into account that the increase of MAFA DNA binding activity may compensate for the overexpression of MAFK in these mice, double transgenic ($MAFA^{-/-}$/$MAFK^{+}$) were generated. However, male and female of these double transgenic mice manifeste a persistent hyperglycemia, and around 5 weeks of age both develop DM, and display the histological features of human diabetic nephropathy, when compared to the $MAFA^{-/-}$ mice. In conclusion, MAFK antagonize the function of MAFA, and its overexpression enhances the diabetic phenotype of $MAFA^{-/-}$ animals (12).

MAFs and cancer

Several observations indicate S-MAFs closely associated with cancer. MAFF is downregulated in several tumor types, and it correlates with clinical outcome. Patients, affected by acute lymphoblastic leukemia and carrying the (12;21) translocation, show decrease of MAFF transcripts when compared to healthy controls (9).

MAFG is amplified in lung adenocarcinoma. Increased MAFG proteins, in cells from smoking patients possibly lead to lung carcinogenesis (13). MAFG is highly expressed in patients with hepatocellular carcinoma (HCC), carrying β-catenin mutations (10), suggesting a proto-oncogene role of MAFG. Patients with familial pancreatic cancer or with chronic myeloid leukemia (CML) often show genetic alterations responsible of MAF proteins dysfunction (10).

In contrast, a lengthening of the survival time has been documented in patients with ovarian and prostate cancer and high expression of MAFF, suggesting that MAFF could act as a tumor suppressor gene.

CML shows SNPs in MAFG sequence, as SNPs in MAFG were found in lung cancers (14). In both cases, the impairment of S-MAF/Nrf2 contributes to the disease onsets and/or progressions, and is often associated with therapeutic resistance. The combination of doxorubicin or taxol or other chemotherapeutic drugs with molecules, masking the Cap 'N' Collar-bZIP domain of Nrf2, could be responsible for an interference of the binding of the complex MAFG/Nrf2 into the promoter of target genes, inducing therapeutic resistance (14).

MAFG shows aberrant methylation in certain cancers. MAFG is overexpressed in colorectal cancer (CRC) and, as heterodimer in conjunction with BACH1, BACH2, and NFE2L1 or DNMT3B, it binds DNA. MAFG Knockdown depresses MLH1 expression. An increased BRAF/MEK/ERK signal, caused by activated BRAF(V600E) molecule, promotes MLH1 transcriptional silencing. The phosphorylation of MAFG at S124, caused by ERK activation, prevents MAFG polyubiquitination, increases its level and DNA binding, and silences transcriptional targets. MAFG may, then contribute to MLH1 repression in BRAF(V600E)-containing CRCs. Therefore, BRAF, as EGFR, are directly responsible for aberrant hypermethylation of many genes in CRCs. (G719S)-mutated EGFR increases RAF/MEK/ERK signaling and MAFG levels, MAFG association with MLH1 promoter, and transcriptionally silencing of MLH1 (15).

This mechanism suggests a general model to favor cancer development, based on tumor-suppressor genes transcriptional repression; and supports the hypothesis that s-MAFs inhibitors may function as new pharmacological targets for cancer treatments.

RNAi analyses reported that MAFG/Bach1 heterodimer captures a chromatin-remodeling factor (CHD8), and the DNA methyl-transferase (DNMT3B) to facilitate hypermethylation and repression of MLH and other tumor suppressors (16).

A study on the whole genome profile analyzed microRNA-218 and mRNA levels on bronchial epithelial cells from smokers and non-smokers. MAFG resulted upregulated in smokers, while mir-218 was decreased in smokers developing lung tumor. This inverse correlation was confirmed when the overexpression of MAFG gene, was induced in mir-218 knockdown bronchial epithelial cell (17).

A recent study showed that insulin-like growth factor-1 (IGF1) induces MAFG transcript in CRC, revealing a potential role for IGF1 in CRC pathogenesis, as suggested for lung cancer (17).

Bile acid homeostasis, MAFs levels and liver cancer

The MAFG expression increases during cholestatic liver injury in mice, in liver of human patients bearing cholestasis, as well cholangiocarcinoma (CCA) and HCC, compared with non-tumor tissues. MAFG upregulation correlates with worse HCC prognosis (10).

MAFG interacts directly, at the E-box elements, with methionine adenosyltransferase a1(MAT1A), and represses its transcription. In spite the relevant role of MAT1A in liver physiology and pathology, mechanisms of its involvement on chronic cholestasis and liver cancers are largely unknown (10).

Decreased hepatic S-adenosylmethionine (SAM) may follow cholestasis, and in turn, the reconstitution of SAM level protects against cholestatic liver injury. SAM, the major biological methyl donor, is synthesized by MATs, enzymes encoded in mammals by two genes, *MAT1A* and *MAT2A*. At least a third gene is involved in the regulation of MAT2A activity. MAT2A catalyzes SAM synthesis ubiquitously, in extra-hepatic tissues (18).

MAT1A is markedly reduced in HCC, and its reactivation in this tumor inhibits tumor growth and metastasis (19,20). Interestingly, low SAM level in hepatocytes, because of loss of MAT1A, is associated to marked c-Myc oncogene induction (10).

Recent data show a connection between s-MAFs and MAT2A. MAT2A could act as a corepressor of MAFK. MAFG can induce MAT2A via IGF1 (21,22). Knockdown of MAFG lowers MAT2A levels and reduces IGF-1-mediated induction of MAT2A. MAT2A overexpression increases MAFG promoter activity, whereas its knockdown reduces this activity (21).

Different bile acids contribute independently to MAT1A or MAT2A levels (22). LCA activates MAFG expression in HepG2 cells, reduces MAT1A expression, but increases that of MAT2A (10,22).

MAFG and MAT2A expression increases, while MAT1A decreases in liver tumors induced, in rats by diethylnitrosamine (10). SAM and UDCA (ursodeoxycholic acid) prevent LCA-mediated increase of MAFG in response to cholestasis, and they exert complementary effects to reduce LCA. Obeticholic acid (OCA), an FXR agonist, increases MAFG, MAT2A and c-MYC expression, but reduces that of MAT1A. It induces cancer cell proliferation, and growth of xenograft tumors in mice. In addition, liver and biliary cancer cells, isolated from mice receiving OCA, undergo to increased growth potential, when incubated in the presence of OCA (23).

In summary, several studies support a role for s-MAFs in human cancer etiopathology and suggest that s-MAFs may be considered as a worthy target gene in cancer therapy.

In conclusion, present knowledge provides evidence that MAF family proteins, interacting with activated oncoproteins or transcriptional regulators, and modulating signaling pathways or protein stability strongly contribute to cellular and tissue behavior under physiological or pathological conditions.

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