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Small Maf proteins (MafF, MafG, MafK): History, structure and function

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Abstract

The small Maf proteins (sMafs) are basic region leucine zipper (bZIP)-type transcription factors. The basic region of the Maf family is unique among the bZIP factors, and it contributes to the distinct DNA-binding mode of this class of proteins. MafF, MafG and MafK are the three vertebrate sMafs, and no functional differences have been observed among them in terms of their bZIP structures. sMafs form homodimers by themselves, and they form heterodimers with cap 'n' collar (CNC) proteins (p45 NF-E2, Nrf1, Nrf2, and Nrf3) and also with Bach proteins (Bach1 and Bach2). Because CNC and Bach proteins cannot bind to DNA as monomers, sMafs are indispensable partners that are required by CNC and Bach proteins to exert their functions. sMafs lack the transcriptional activation domain; hence, their homodimers act as transcriptional repressors. In contrast, sMafs participate in transcriptional activation or repression depending on their heterodimeric partner molecules and context. Mouse genetic analyses have revealed that various biological pathways are under the regulation of CNC-sMaf heterodimers. In this review, we summarize the history and current progress of sMaf studies in relation to their partners.

1. Introduction

It has been over two decades since the small Maf (musculoaponeurotic fibrosarcoma) proteins (sMafs) were isolated (Fujiwara et al., 1993). sMafs are small transcription factors with sizes of approximately 160 amino acids or approximately 18 kDa. sMafs have been diversified into three functionally redundant factors that can form homodimers or heterodimers with various partners. Many lines of inquiry have revealed the importance of sMafs in various biological pathways. Interestingly, the functions of sMaf proteins vary depending on their partners and context. To comprehensively understand sMaf-mediated

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gene regulation, we and other groups have conducted various molecular and cellular analyses. Here, we summarize the current information related to sMaf functions.

2. Identification of sMaf genes

sMafs are basic region leucine zipper (bZIP)-type transcription factors belonging to the Maf family. Although the Maf family and other bZIP-type transcription factors, such as the AP-1 family, share structural similarities, their functions are markedly diversified. The founding member of the Maf family is c-Maf, which was cloned as a cellular counterpart of v-Maf oncogene isolated from avian musculoaponeurotic fibrosarcoma (Nishizawa et al., 1989). Three other members in addition to c-Maf, *i.e.*, MafA, MafB and NRL, constitute the large Maf family (Swaroop et al., 1992; Kataoka et al., 1994; Benkhelifa et al., 1998) (Fig. 1).

Two additional factors, MafK and MafF, were isolated in a low-stringency cDNA library screen using the v-Maf gene (Fujiwara et al., 1993). MafG was later isolated in a separate screening (Kataoka et al., 1995). Though each of the large Maf proteins has a transcriptional activation domain, MafF, MafG and MafK lack any canonical transcriptional activation domains, and based on their small size, they have been classified as the small Maf (sMaf) family (Fig. 1). The official full gene names of *MafF*, *MafG* and *MafK* are “v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog F, G, and K”, respectively.

Before the sMaf genes were cloned, a protein complex binding to a specific motif in the human β -globin locus control region (LCR) and other erythroid-related regulatory regions was identified as nuclear factor erythroid 2 (NF-E2) (Mignotte et al., 1989a; Mignotte et al., 1989b; Romeo et al., 1990). Later, a 45 kDa subunit of NF-E2 (p45 NF-E2) was purified and cloned (Andrews et al., 1993a; Ney et al., 1993). The p45 subunit was identified as a bZIP factor, but p45 alone could not bind to the NF-E2-binding motif (Andrews et al., 1993b). NF-E2 was finally identified as a heterodimeric protein complex comprising p45 and another bZIP factor, sMaf (Igarashi et al., 1994). This heterodimer was found to bind to the NF-E2 motif.

MafF, MafG, and MafK are conserved among vertebrates, including human, mouse, rat, chicken and zebrafish. Zebrafish have four sMafs: two MafGs (Mafga and Mafgb); MafK; and MafF, which was originally cloned as MafT (Takagi et al., 2004). In fruit fly, Maf-S was identified as the only sMaf (Veraksa et al., 2000). Therefore, it is assumed that MafF, MafG, and MafK originated from two rounds of whole genome duplication during molecular evolution.

3. Gene structure and expression profile of sMafs

In human and mouse, the genes encoding sMafs (*sMaf* genes) are composed of three exons. It has been reported that all mouse *sMaf* genes harbor alternatively used multiple first exons (Motohashi et al., 1996; Onodera et al., 1999; Katsuoka et al., 2005a), and according to the NCBI Reference Sequence (RefSeq) and UCSC EST databases, all human *sMaf* genes also have multiple alternative first exons. *MafF*, *MafG* and *MafK* are expressed broadly in various tissues, but each *sMaf* gene has a distinct expression profile (Toki et al., 1997; Onodera et al., 1999). A recent transcriptome sequencing analysis also supports that each

sMaf gene exhibits a broad but distinct expression profile (Petryszak et al., 2013). Mechanisms underlying the differential expression of *sMaf* genes have been well studied in mice, as follows.

In the mouse *MafK* gene, two alternative first exons have been identified as follows: the IM exon is broadly utilized, whereas the IN exon is specifically utilized in neural tissues (Fig. 2) (Motohashi et al., 1996; Motohashi et al., 1998). In the 3' region of *MafK*, there is an enhancer referred to as Hematopoietic and Cardiac Enhancer for MafK (HCEK), which drives *MafK* expression in both hematopoietic and cardiac tissues (Katsuoka et al., 2000). Hematopoietic GATA factors (GATA1 or GATA2) and cardiac GATA factors (GATA4 or GATA6) bind to GATA motifs in HCEK and regulate *MafK* gene expression in specific tissues. It has also been shown that NGF (Torocsik et al., 2002), TGF- β (Okita et al., 2013), AKT (Ro et al., 2010) and Wnt signaling (Wang et al., 2015) influence *MafK* gene expression.

In mice, three alternative first exons of *MafG* have been identified (Ia, Ib and Ic; Fig. 2) (Katsuoka et al., 2005a). In the promoter proximal region of Ic exon, there is an antioxidant response element (ARE) to which the Nrf2-sMaf heterodimer binds (Katsuoka et al., 2005a) (see details in Chapter 5). An important observation is that *MafG* is induced by Nrf2 inducers, such as electrophilic chemicals, through the ARE (Crawford et al., 1996; Katsuoka et al., 2005a). Certain chemicals have also been reported to induce the expression of the *MafK* and *MafF* genes (Moran and Mulcahy, 2002). *MafG* is also induced by the Farnesoid X receptor (FXR) through a FXR response element (de Aguiar Vallim et al., 2015)(Fig. 2). The three first exons of the *MafG* gene are clustered in a relatively compact region, and the 6.8 kb upstream region, which includes these three exons and the 3.3 kb downstream region of this gene, are able to recapitulate endogenous mouse *MafG* expression in transgenic reporter mice *in vivo*. Therefore, this region has been referred to as the MafG Regulatory Domain (MGRD) (Yamazaki et al., 2012). The MGRD retains stable, strong enhancer activity when employed in mice *in vivo* (Yamazaki et al., 2012), and it has been used for transgenic overexpression or transgenic complementation rescue analyses (Yamazaki et al., 2012; Hirotsu et al., 2014; Murakami et al., 2014).

Similarly, in mice, the three alternative first exons of *MafF* have been identified (Ia, Ib and Ic; Fig. 2) (Onodera et al., 1999). Another first exon, which is located downstream of Ic (tentatively referred to here as Id; Fig. 2) is registered in the RefSeq database. Although the RefSeq data show that Ic and Id include alternative start codons that produce alternative protein isoforms, the functional significance of either isoform has not yet been validated. *MafF* is induced by proinflammatory cytokines in myometrial cells (Massrieh et al., 2006), and it is upregulated in the livers of diet-induced obesity mouse models (Kim et al., 2015).

4. Protein structure of sMafs

Many domains or motifs have been recognized in the small 18 kDa structures of sMafs (Fig. 3). sMafs harbor a basic region that functions as the DNA-binding motif. The sMafs also harbor a leucine zipper structure that is required for dimer formation with themselves and with other bZIP transcription factors. The elaborate dimer formation involved in the

meticulous gene regulation based on this structure is described in Chapter 5. Each sMaf also retains an extended homology region (EHR), which is a domain conserved among the sMaf and large Maf proteins (Kusunoki et al., 2002) that contributes to stable DNA binding. X-ray crystallographic analysis has determined the structure of the MafG-DNA complex (Kurokawa et al., 2009). MafG consists of three helices (H1, H2, and H3). H1, H2, and the beginning of H3 comprise the EHR, and the remaining portion of H3 includes the bZIP region (Fig. 4A). The basic region in H3 fits into the major groove of DNA, and H1 and H2 interact with an N-terminal region of H3 from the outside (Fig. 4B). A tyrosine residue in the basic region of MafG contributes to the unique DNA-binding mode of Maf, as detailed in chapter 5.1.

Similar to other bZIP factors, the basic region of sMafs includes a nuclear localization signal (LaCasse et al., 1993). In addition, the C-terminal region of each sMaf contains a nuclear matrix-targeting signal (Motohashi et al., 2011). The C-terminal region is required for localization of the p45 NF-E2-sMaf heterodimer to the subnuclear domain, where proper transcription can be facilitated. Although sMafs are primarily localized in the nucleus, extranuclear localization of sMafs was reported in Hepatitis C virus-replicating cells (Carvajal-Yepes et al., 2011). It has been suggested that extranuclear sMafs can result in mislocalization of Nrf2 and thereby inhibit the induction of Nrf2 target genes.

Post-translational modifications have also been reported for MafG. A sumoylation consensus motif (Ψ KXEX) is found in the N-terminal region and is required for sMaf homodimer-mediated repression (Motohashi et al., 2006). In mice, a significant amount of endogenous MafG is conjugated with SUMO-2/3 in bone marrow cells. The C-terminal region of MafG contains an ERK phosphorylation site (Fang et al., 2014), and phosphorylation of this site has been reported to stabilize MafG by inhibiting its ubiquitination.

5. Dimer formation and DNA binding of sMafs

5.1. sMaf Homodimers

An interesting feature of sMafs is that they form homodimers by themselves (Kataoka et al., 1995). Because no functional differences have been reported between the bZIP domains of sMafs, it is assumed that any combination of sMafs can form homodimers (e.g., MafK-MafG and MafK-MafF). The consensus binding motif for sMaf homodimers are palindromic (TGCTGACTCAGCA); this motif is known as the Maf recognition element (MARE) (Fig. 5A). This element is also defined as T-MARE (MARE containing a TRE (TPA-responsive element: TGACTCA or AP-1-binding site) in its core)(Fig. 5A).

An important finding here is that unlike AP-1 factors, the basic regions of Maf factors recognize the flanking GC boxes of MARE in addition to the core region. The basic region of a Maf family protein possesses a specific tyrosine residue (Tyr64, Fig. 3) that is critical for the recognition of the GC box (Kimura et al., 2007) (Fig. 5); this position is usually substituted by alanine residues in other bZIP factors (Fig. 5). A crystallographic analysis determined that Tyr64 does not interact directly with the GC box but affects the side-chain orientation of Arg57 and Asn61 (Fig. 2); these two residues are not unique to sMafs but critical for the recognition of GC (Kurokawa et al., 2009). Although the EHR domain of a

sMaf does not contact the DNA directly, the domain supports the recognition of specific DNA sequences by sMafs (Kurokawa et al., 2009). sMafs are known to bind not only to T-MARE but also to palindromic (TGCTGACGTCAGCA) motifs, which are defined as C-MARE (MARE-containing CRE (cAMP-responsive element: TGACGTCA) motif) (Kataoka et al., 1995) (Fig. 5A). However, the binding of sMaf to C-MARE has not been studied extensively.

Because sMafs lack any transcriptional activation domains, sMaf homodimers act as transcriptional repressors (Motohashi et al., 2000). It has been reported that sumoylation at Lysine 14 (K14) within the sMaf sumoylation motif is required for sMaf homodimer-mediated repression (Motohashi et al., 2006). Although the overexpression of wild-type MafG in bone marrow represses its target gene expression, the overexpression of sumoylation-deficient MafG (K14 is mutated to arginine) fails to repress target gene expression. One plausible explanation for this observation is that this is an active repression rather than passive repression, in which the homodimer simply occupies the binding site because a HDAC inhibitor blocks the sMaf homodimer-mediated repression (Motohashi et al., 2006).

5.2 Heterodimers with CNC/Bach transcription factors

sMafs form heterodimers with CNC transcription factors, p45 NF-E2, and NF-E2-related factors (Nrf1, Nrf2 and Nrf3) and with the Bach (BTB and CNC homology) family transcription factors (Bach1 and Bach2), which are distantly related to the CNC family (Fig. 5B) (Igarashi et al., 1994; Oyake et al., 1996; Itoh et al., 1997; Johnsen et al., 1998; Kobayashi et al., 1999). The approved gene symbols for the CNC factors are *NFE2*, *NFE2L1*, *NFE2L2* and *NFE2L3*. To avoid any confusion, we wish to emphasize here that *NFE2L1* and *NFE2L2* (encoding Nrf1 and Nrf2) are different molecules than the nuclear respiratory factors *Nrf1* and *Nrf2* (Scarpulla, 1997). Because CNC and Bach proteins cannot efficiently bind to DNA by themselves, sMafs are obligatory binding partners. Notably, the CNC homolog in *C. elegans*, Skn-1, lacks the leucine zipper domain and acts as a monomer (Blackwell et al., 1994) (Fig. 5B). Thus, we surmise that during the course of molecular evolution, the CNC and Bach factors acquired sMafs as indispensable partners to perform higher order transcriptional regulation through the formation of heterodimers (Fig. 5B).

Intriguingly, we can identify several consensus sequences that bind CNC-sMaf heterodimers in various biological contexts. As described above, the original NF-E2 motif “RTGASTCAGCR” was reported as the binding site for the p45 NF-E2-sMaf heterodimer. Another example is the antioxidant/electrophile response element (ARE/EpRE) “RGTGACNNNGC”, which is found in the regulatory regions of genes encoding phase II drug metabolizing enzymes and antioxidant enzymes (Friling et al., 1990; Rushmore et al., 1991). ARE/EpRE is subsequently recognized as the binding site for Nrf2-sMaf heterodimers (Itoh et al., 1997).

Recent ChIP-Seq analyses of the binding sites for the Nrf2-, p45-NF-E2-, and Bach1-sMaf heterodimers revealed that the representative binding motifs for these heterodimers are indeed “RTGACTCAGCA” (Hirotsu et al., 2012b; Fujita et al., 2013; Warnatz et al., 2011) (Fig. 5A). A common feature of these motifs is that the TRE-core is located at the center,

with a flanking sequence of R (A or G) on one side and a GC sequence (or GC box) on the other side. The R flanking the TRE core is known to reduce sMaf binding (Kimura et al., 2007). Upon binding of CNC-sMaf heterodimers, CNC factors recognize the R side and sMaf recognizes the GC box side. We previously proposed that the CNC-sMaf heterodimer preferentially binds the CNC-sMaf-binding element (CsMBE) “RTGA(C/G)NNNGC” (Otsuki et al., 2016), thus discriminating this element from the elements that preferentially host Maf homodimers (MARE) (Fig. 5B). Nonetheless, some Nrf2-sMaf-binding sites harbor GC boxes on both sides of the TRE-core, allowing the binding of sMaf homodimers (Fig. 5B) (Otsuki et al., 2016). We surmise that both CNC-sMaf heterodimers and sMaf homodimers participate in the transcriptional regulation of genes through these sites. Indeed, several studies have suggested a switch between CNC-sMaf heterodimers and sMaf homodimers (Motohashi et al., 2000; Yang et al., 2010).

5.3 Heterodimers with other bZIP transcription factors

In addition to CNC and Bach factors, it has been reported that sMafs form heterodimers with other bZIP transcription factors, such as c-Fos and c-Maf (Kataoka et al., 1995; Dhakshinamoorthy et al., 2002). A comprehensive interaction analysis of 49 bZIP transcription factors also showed that sMafs have an affinity to several bZIP factors, including c-Jun and ATF7 (Newman and Keating, 2003). However, the biological significance of these sMaf-containing heterodimers is currently unclear, and further studies are needed to clarify their contribution.

6. *In vivo* roles of sMafs revealed by mouse genetic studies

To examine the *in vivo* function of sMafs, *MafF*, *MafG* and *MafK*, knockout mice were generated (Shavit et al., 1998; Onodera et al., 1999; Onodera et al., 2000). Though *MafF* and *MafK* knockout mice show no apparent phenotype, *MafG* knockout mice exhibit mild phenotypes (see chapter 6.1). Therefore, the phenotypic analyses of *sMaf* double and triple compound knockout mice were conducted to delineate the functions of sMafs *in vivo*. Because CNC proteins cannot bind to DNA and exert their functions in the absence of sMafs, it seems reasonable to deduce that the phenotypes observed in *sMaf* mutant mice will also be observed in CNC mutant mice (Fig. 6A). Indeed, available lines of evidence demonstrate that the substantial phenotypes observed in *sMaf* mutant mice are commonly observed in CNC mutant mice. The phenotypes observed in *sMaf* mutant mice are summarized in Fig. 6B and discussed below.

6.1. Loss of function of p45 NF-E2-sMaf heterodimers

A founding member of the CNC family of transcription factors is p45 NF-E2, which is specifically expressed in hematopoietic lineages (Andrews et al., 1993a). Indeed, p45 NF-E2 knockout mice show abnormal megakaryocyte differentiation and thrombocytopenia (Shivdasani et al., 1995). In agreement with this observation, *MafG* knockout mice show similarly abnormal megakaryocyte differentiation and thrombocytopenia (Shavit et al., 1998), whereas *MafG*^{-/-}::*MafK*^{-/-} compound mutant mice suffer from much more severe thrombocytopenia (Onodera et al., 2000). A ChIP-Seq analysis of p45 NF-E2-binding sites revealed that p45 NF-E2 binds to a set of genes that are critical for platelet function and

production (Fujita et al., 2013). Surprisingly, it has been reported that the overexpression of p45 NF-E2, MafG and MafK can differentiate mouse and human fibroblasts into megakaryocytes (Ono et al., 2012). These results demonstrate that p45 NF-E2-sMaf heterodimers are critical regulators of megakaryocyte differentiation.

It has been reported that p45 NF-E2-sMaf heterodimers regulate β -globin gene expression (Igarashi et al., 1995; Sawado et al., 2001). In the far upstream region of the β -globin gene, there is a LCR composed of several DNase I hypersensitive sites harboring NF-E2 motifs (Raich et al., 1990; Talbot et al., 1990). Overexpression and knockout analyses of p45 NF-E2 using leukemic cell lines showed the involvement of p45 NF-E2-sMaf heterodimers in β -globin gene regulation (Lu et al., 1994; Kotkow and Orkin, 1995). However, mouse genetic studies have failed to show the importance of both p45 NF-E2 and sMafs in β -globin gene regulation (Shivdasani and Orkin, 1995; Onodera et al., 2000), suggesting possible compensation by other CNC factors.

6.2. Loss of function of Nrf1 -sMaf heterodimers

In addition to megakaryocytic phenotypes, *MafG* knockout and *MafG::MafK* compound knockout mice exhibit neurological phenotypes. *MafG* knockout mice show abnormal behavior, such as limb grasping and impaired coordination (Shavit et al., 1998; Onodera et al., 2000). *MafG*^{-/-}::*MafK*^{+/-} mice show much more severe neurological phenotypes than *MafG* single knockout mice, such as ataxia accompanied by progressive neuronal degeneration (Katsuoka et al., 2003). These phenotypes are accelerated in *MafG*^{-/-}::*MafK*^{-/-} mice, which cannot survive until weaning (Onodera et al., 2000). Importantly, similar neurological phenotypes were also observed in central nervous system (CNS)-specific *Nrf1* knockout mice (Kobayashi et al., 2011; Lee et al., 2011), supporting the notion that Nrf1-sMaf heterodimers are critical for neuronal homeostasis. However, the target genes regulated by Nrf1-sMaf heterodimers in the CNS remain to be fully elucidated.

In addition, conditional liver-specific *Nrf1* knockout mice suffer from steatohepatitis (Xu et al., 2005; Ohtsui et al., 2008). However, because *MafG*^{-/-}::*MafK*^{-/-} mice die before weaning (Onodera et al., 2000), this phenotype has not been confirmed in sMaf knockouts. *Nrf1* knockout mouse studies have suggested that Nrf1 regulates genes involved in various metabolic pathways, such as fatty acid and amino acid metabolism, as well as proteasome subunit genes (Hirotsu et al., 2012a). These genes are thought to be regulated by Nrf1-sMaf heterodimers, and further studies, such as sMaf conditional targeting analyses, are expected to support this hypothesis.

6.3. Loss of function of Nrf2-sMaf heterodimers

Nrf2 is a master regulator of antioxidant and xenobiotic metabolizing enzyme genes (Itoh et al., 1997). Under normal unstressed conditions, Nrf2 is primarily ubiquitinated by the Keap1 E3-ubiquitin ligase complex and degraded through the proteasome system. For details regarding the degradation mechanisms for Nrf2, please refer to the review papers (Harder et al., 2015; Suzuki and Yamamoto, 2015). In response to oxidative and electrophilic stresses, however, Nrf2 is not ubiquitinated by the Keap1 E3-ubiquitin ligase complex but is instead stabilized and imported into the nucleus (Cullinan et al., 2004; Kobayashi et al., 2004). Nrf2

heterodimerizes with sMafs to activate specific target genes through ARE/EpRE (Friling et al., 1990; Rushmore et al., 1991). *Nrf2* knockout mice show impairment in the oxidative/xenobiotic stress response (Itoh et al., 1997). Recent analyses have also demonstrated that Nrf2 regulates genes involved in glucose metabolism and NADPH production (Mitsuishi et al., 2012).

The first genetic evidence supporting the biological significance of Nrf2-sMaf heterodimers was provided by compound mutant analyses of Keap1 and sMaf mice (Motohashi et al., 2004). Keap1 mutant mice show postnatal lethality due to hyperactivation of Nrf2, which results in hyperproliferation of keratinocytes in the esophagus and forestomach (Wakabayashi et al., 2003). These phenotypes are negated not only by the simultaneous disruption of Nrf2 (Wakabayashi et al., 2003) but also by the disruption of MafG and MafF (Motohashi et al., 2004). Conclusive evidence was provided by the genetic analyses of *sMaf* triple compound mutant mice (Katsuoka et al., 2005b). In embryonic fibroblasts derived from *sMaf* triple knockout embryos, the induction of Nrf2-regulated cytoprotective genes is severely impaired. Recent ChIP-Seq analyses of Nrf2-MafG-binding sites support the notion that Nrf2-sMaf heterodimers globally contribute to the regulation of antioxidant and metabolic networks (Hirotsu et al., 2012b).

6.4. Loss of function of Bach-sMaf heterodimers

Bach1 is a negative regulator of heme oxygenase-1 (Hmox1) (Oyake et al., 1996; Sun et al., 2002). The *Hmox1* gene has two distant enhancers harboring multiple AREs through which Bach1 represses gene transcription. Heme inhibits Bach1 binding to the enhancers, leading to the derepression of the *Hmox1* gene (Sun et al., 2002). *Bach1* knockout mice show derepression of the *Hmox1* gene in various tissues. In agreement with this observation, derepression of the *Hmox1* gene is also observed in brains of *MafG^{-/-}::MafK^{+/-}* mice (Katsuoka et al., 2003) and fibroblasts from *sMaf* triple knockout mice (Katsuoka et al., 2005b). These genetic data support the role of Bach1-sMaf heterodimers as negative regulators of the *Hmox1* gene.

Similarly, Bach2-sMaf heterodimers are known to negatively regulate the immunoglobulin heavy chain gene through MAREs in the 3' enhancer region (Muto et al., 1998). Bach2-deficient mice exhibit defects in antibody class switching and somatic hypermutation in B cells (Muto et al., 2004). However, these B-cell phenotypes remain to be examined in murine sMaf mutant lines to demonstrate that sMafs are the indispensable partner molecules of the Bach2 factor.

6.5. Other phenotypes observed in sMaf mutant mice

Lens defects progressively developing into cataracts were reported in *MafG^{-/-}::MafK^{+/-}* mice (Agrawal et al., 2015). Though microarray analyses revealed several candidate genes that account for lens phenotypes, it is unclear which CNC factors act as partners of sMafs in this context.

sMaf triple knockout mice are embryonic lethal, indicating that sMafs are indispensable for embryo development (Yamazaki et al., 2012). Although mutant embryos grow normally until embryonic day (E) 9.5, the embryos show growth retardation and fetal liver hypoplasia,

and they die around E13.5. Apoptotic cells are observed in fetal livers of *sMaf* triple knockout mice. Because *Nrf1::Nrf2* double knockout mice are also embryonic lethal around E13.5 (Leung et al., 2003), the deficiency of both Nrf1-sMaf and Nrf2-sMaf heterodimers may elicit the lethality of *sMaf* triple knockout mice.

6.6. sMaf functions revealed by overexpression analyses

To examine the functions of sMafs *in vivo*, several overexpression analyses of sMafs or their mutants have been conducted. The overexpression of dominant negative MafK in pancreatic β -cells promotes MafA binding to MARE in the promoter region of the *insulin-2* gene by inhibiting endogenous sMaf binding (Nomoto et al., 2015), which suggests potential competition between sMaf and large Maf proteins for *insulin-2* gene regulation. Adenovirus-mediated overexpression of MafG represses *Cyp8b1* and several genes involved in bile acid synthesis, suggesting the negative contribution of sMafs to those genes (de Aguiar Vallim et al., 2015).

7. sMaf functions and diseases

As described in chapter 6 and summarized in Table 1, loss-of-function of sMafs results in various disease phenotypes, such as progressive neuronal degeneration, cataracts, thrombocytopenia and embryonic lethality. In addition, many studies have reported relationships between sMAFs and human diseases.

7.1. Involvement of sMafs in disease prevention

Several SNPs in *MAFF* and *MAFG* were found to be associated with chronic myeloid leukemia (Martínez-Hernández et al., 2014), and SNPs in *MAFG* were found to be associated with lung cancers (Wang et al., 2010). In both cases, it was suggested that impairment of Nrf2-sMaf heterodimer function contributes to the disease onsets and/or progressions. The links between the Nrf2-Keap1 system and diseases have been extensively studied. Impaired Nrf2 functions result in the dysregulation of antioxidant and xenobiotic metabolism and an increased susceptibility to various stimuli, including exogenous chemicals (e.g., acetaminophen) and endogenous factors (e.g., electrophilic compounds and reactive oxygen species) (Enomoto et al., 2001; Cho et al., 2015). In addition, several lines of evidence have revealed the contribution of Nrf2 to anti-inflammatory responses. Impaired Nrf2 functions result in prolonged inflammation, which is associated with various disorders, including neurodegeneration and arteriosclerosis (Mimura and Itoh, 2015; Yamazaki et al., 2015). Although direct evidence has not been provided, it is likely that sMAFs are involved in disease prevention together with NRF2.

7.2. sMafs and cancer

The involvement of sMaf in tumor onset and progression is complicated. Because Nrf2-sMaf heterodimers function as critical regulators of antioxidant and xenobiotic metabolism, impairment of the heterodimers increases the risk of various cancers. Conversely, many SNPs in *NRF2* and *KEPA1* that result in constitutional activation of Nrf2, are observed in many tumors (Suzuki and Yamamoto, 2015). Cancer cells exploit Nrf2-sMaf heterodimers to

produce reducing power, which is required for cell survival and cell cycle progression (Mitsuishi et al., 2012).

MafG is involved in aberrant hypermethylation in certain types of cancers. Constitutively activating mutations in the BRAF oncoprotein promote proliferation and malignant transformation (Dhomen and Marais, 2007). Recently, an RNAi screening study reported that the Bach1-MafG heterodimer recruits a chromatin-remodeling factor (CHD8) and a DNA methyl-transferase (DNMT3B) to facilitate the hypermethylation and repression of the mismatch repair gene *MLH1* and other tumor suppressor genes harboring CpG island promoters (Fang et al., 2014). Considering these observations, inhibitors for sMafs may be new pharmacological targets for cancer treatments.

8. Conclusion

In summary, accumulating lines of evidence unequivocally demonstrate that sMafs play roles as critical regulatory hubs for the CNC-sMaf transcription factor network. Understanding the cross-talk among sMaf-containing hetero- and homodimers is especially important for answering the question of how higher organisms have adopted the dimer-mediated transcription factor network. Further analyses, including proteome, transcriptome, and cistrome analyses, will provide an in-depth understanding of the cellular dynamics of sMafs and their partners, thus yielding more comprehensive insights into sMafs.

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Highlights

- The small Maf (sMaf) proteins are bZIP-type transcription factors.
- sMaf proteins form homodimers and heterodimers with CNC and Bach proteins.
- sMaf proteins participate in activation or repression depending on their partners.
- Various biological pathways are under the regulation of sMaf-containing dimers.

Fig. 1.

Structures of the Maf family proteins and other bZIP-type transcription factors. c-Maf, MafA, MafB and NRL constitute the large Maf family. MafG, MafK and MafF constitute the small Maf family. c-Jun and c-Fos are members of the AP-1 family. The protein regions are indicated by different colors: the transcriptional activation domain (TAD; green), extended homology region (EHR; red), CNC domain (orange), basic region (purple) and leucine zipper (blue). The lengths of amino acid sequences for human bZIP factors and *C. elegans* Skn-1 are provided from the NCBI database (NP_002219, NP_005243, NP_005351, NP_963883, NP_005452, NP_006168.1, NP_002350, NP_002351, NP_001155044, NP_001129495, NP_741404)

Fig. 2.

Structures of mouse *mafG*, *mafK*, and *mafF* genomic loci. The first exons are indicated by blue boxes. The other exons are indicated by red boxes. Bent arrows indicate transcription from promoters. The first exons are differentially retained in different splice isoforms. Known cis-regulatory regions, the FXR-binding region, the Nrf2-sMaf-binding region and the hematopoietic and cardiac enhancer for MafK (HCEK) region are indicated by open circles with the names of transcription factors acting through these regions.

Amino acid sequence alignments of human, mouse and chicken sMafs. SUMO consensus motifs, extended homology regions, basic regions, leucine zipper leucine residues, and phosphorylation sites are indicated by boxes. Amino acid residues not conserved among individual sMafs are underlined in the chicken sequences. MafF is less conserved among species. Tyr64 in red is conserved among basic regions of the small and large Maf families but not in the other bZIP factors. Arg57 and Asn61 in blue are not unique to the Maf family but critical for GC binding.

Fig. 4. Structure of the MafG homodimer binding to DNA. Two different views (A and B) of the crystal structure of the MafG homodimer and DNA are shown (Protein Data Bank (PDB) Code 3A5T). A symmetry axis is indicated as a red pole in the center of MafG homodimers. Oligonucleotide sequences are shown at each location.

Fig. 5.

Cis-acting consensus sequences recognized by CNC and sMaf. (A) Alignment of the CRE, C-MARE, TRE, T-MARE, NF-E2, and ARE/EpRE motifs. *De novo* motifs revealed by ChIP-Seq studies (Nrf2, p45NF-E2, and Bach1) are shown below [modified data from (Hirotsu et al., 2012b; Warnatz et al., 2011; Fujita et al., 2013) are shown]. (B) Binding mode of Skn-1, CNC, Bach, and sMaf proteins. Although Skn-1 binds to DNA as a monomer (left box), CNC and Bach proteins bind to DNA as heterodimers with sMafs (right box). Although CNC-sMaf and Bach-sMaf heterodimers preferentially bind to CsMBE, sMaf homodimers preferentially bind to MARE. Asterisk: the exact binding sequences for Jun-sMaf and Fos-sMaf heterodimers have not yet been determined.

Fig. 6.
Genetic analyses of CNC, Bach, and sMaf mutant mice. (A) In the absence of sMaf, CNC and Bach proteins cannot bind to DNA to exert their functions. It is expected that phenotypes observed in *CNC* and *Bach* mutant mice are also observed in *sMaf* mutant mice. (B) Summary of phenotypes observed in *sMaf* single, double, and triple knockout mutant mice. Note that the phenotype observed in the *CNC* and *Bach* mutant mice are mostly observed in *sMaf* mutant mice depending on the magnitudes of sMaf loss-of-functions. When similar phenotypes are reported in the analyses of *CNC* or *Bach* mutant mice, the compositions of CNC-sMaf heterodimers are shown with their illustrations.

Table 1

Phenotypes and diseases associated with CNC/Bach-sMaf functions

Site	Phenotypes and Diseases	References
CNS	Progressive neuronal degeneration	Mouse Genetics (<i>Nrf1</i> , Kobayashi et al., 2011; Lee et al., 2011)
		Mouse Genetics (<i>MafG</i> , <i>MafK</i> , Onodera et al., 2000; Katsuoka et al., 2003)
Eye	Cataract	Mouse Genetics (<i>MafG</i> , <i>MafK</i> , Agrawal et al., 2015)
Liver	Steatohepatitis	Mouse Genetics (<i>Nrf1</i> , Ohtsuji et al., 2008)
	Hepatotoxicity (e.g., Acetaminophen)	Mouse Genetics (<i>Nrf2</i> , Enomoto et al., 2001)
Hematopoietic	Thrombocytopenia	Mouse Genetics (<i>p45 NFE2</i> , Shivdasani et al., 1995)
		Mouse Genetics (<i>MafG</i> , <i>MafK</i> , Shavit et al., 1998; Onodera et al., 2000)
	Delays in hematopoietic recovery	Mouse Genetics (<i>MafG</i> , Li et al., 2010)
	Chronic myeloid leukemia	SNPs (<i>MAFG</i> and <i>MAFF</i> , Martínez- Hernández et al., 2014)
Embryogenesis	Growth Retardation/Lethality	Mouse Genetics (<i>Nrf1</i> , <i>Nrf2</i> , Leung et al., 2003)
		Mouse Genetics (<i>MafF</i> , <i>MafG</i> <i>MafK</i> Yamazaki et al., 2012)
Tumorigenesis	Tumor suppression	
		Mouse Genetics (<i>Nrf2</i> , Iida et al., 2007; Bauer et al., 2011)
		SNPs (<i>NRF2</i> Suzuki et al., 2013; Review Paper: Cho et al., 2015)
		SNPs (<i>MAFG</i> Wang et al., 2010)
	Tumor progression	Somatic Mutation (<i>NRF2</i> , <i>KEAP1</i> , Review Paper: Cho et al., 2015)
		siRNA Knockdown (<i>Bach1</i> , <i>MafG</i> Fang et al., 2014)