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Review

In vivo modulation of HMGA2 expression

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ABSTRACT

While the biochemical role of the HMGA proteins has largely been elucidated in tissue culture, the majority of the insight as to their physiological functions in the processes of proliferation and development has been established in animal models of overexpression (transgenic) and null mice (knockouts). An emphasis has been placed on the HMGA2 studies which have defined its critical role in mesenchymal proliferation and differentiation.

1. Introduction: The HMGA proteins

Cell proliferation and differentiation are highly coordinated processes during development [1], and require the precise regulation of gene expression. One process that facilitates the orchestration of these changes in gene expression patterns is remodeling of chromatin structure, which in turn modulates the interaction of transcription regulatory proteins with DNA. These three-dimensional changes in chromatin structures are effected by so-called "architectural transcription factors." The high mobility group (HMG) proteins are abundant heterogeneous, nonhistone components of chromatin [2] that act as such architectural factors [3] contributing to transcriptional regulation. The members of the HMGA family of proteins, HMGA1a, HMGA1b, HMGA1c and HMGA2, each contain three N-terminal "AT-hook" motifs, through which they bind preferentially to AT-rich sequences in B-form DNA, and induce conformational changes to promote the recruitment of transcription factors in a complex termed the "enhanceosome." In addition to the AT-hook domains, the HMGA family members also have an acidic tail, which may be important for protein–protein interaction and for the recruitment of specific proteins to the enhanceosome.

Several lines of evidence, taken together, show that the HMGA proteins play a central role in growth regulation and cellular proliferation. For example, Hmga protein expression is rapidly induced in quiescent normal cells following exposure to growth stimulatory factors, thus classifying the HMGA proteins as 'delayed early response genes' [4]. *In situ* hybridization analysis of both *Hmga1* and *Hmga2* showed that they are expressed during mouse embryogenesis. *Hmga1* expression was observed from 10.5 days post coitum (dpc) to 16.5 dpc whereas *Hmga2* was expressed from 10.5 to 15.5 dpc [5]. Furthermore, in normal development, *Hmga2* expression has been limited to the mesenchyme [6]. Thus, expression of the HMGA proteins is essentially extinguished upon the overt differentiation of the embryonic mesenchyme. However, while HMGA2 expression is not detected in normal adult tissues, low level of HMGA1 expression has been observed in adult tissues of both humans and mice [7,8] suggesting a function in the differentiated cells of adult tissues. The expression of Hmga1 and Hmga2 has also been observed in neoplastic cells (reviewed in [9]). For example, while not acting as traditional oncogene *per se*, it has been shown that Hmga2 expression is essential for the viral neoplastic transformation of rat thyroid cells.

2. *Hmga2* null mice

The characterization of the spontaneous mouse mutant *pygmy* and the transgenic insertional mutant, mini-mouse, revealed that both mutants had a similar phenotype [10,11]. A phenotypic analysis performed on homozygous mutants at the *pygmy* locus showed that the adults are 40% of the size of wild-type litter-mates whereas adult heterozygotes are 80% of the size of wild-type litter-mates. Further analysis of the various organs showed that, in general, there was a reduction in weight of each organ commensurate with the overall reduction in body size. However, two organs did not follow this pattern, the brain being disproportionately larger and the adrenals disproportionately smaller in the mutant mice [10,11].

Both mini-mice and *pygmy* mice had large regions of deletions at the *pygmy* locus [11]. *Hmga2* was identified as a gene that was deleted in both strains [5]. In order to confirm that the phenotype of these mice was solely attributable to the absence of the *Hmga2* gene, Zhou

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et al. [5] created a targeted knock-out mouse that lacked exons 2 and 3 of *Hmga2* referred to as the *Hmga2* null mouse ($Hmga2^{-/-}$). They showed that the $Hmga2^{-/-}$ mice had the same phenotype that was observed in the *pygmy* mice, and that the growth retardation in these mice resulted from a decrease in mesenchymal cell proliferation [5].

3. Hmga2 and adipogenesis

An intriguing aspect of the *Hmga2* null mice was that they had significantly less adipose tissue than their wild-type or heterozygous litter-mates [5,10,11]. Upon gross histological examination, the adipose tissue of $Hmga2^{-/-}$ mice appeared normal and the cellular distribution was similar to that of adipose tissue from wild-type mice, thus the decrease in adipose depot mass was due to a decrease in the total number of cells in the fat pads [12]. Concomitant research revealed that the *HMGA2* gene is frequently disrupted in human solid tumors of a differentiated mesenchymal origin such as lipomas, pleomorphic adenomas of the salivary gland, uterine leiomyomas, and lung hamartomas [13–18] and that these tumors and others often have chromosome 12 rearrangements, which directly affect the *HMGA2* gene [13–15]. Since adipocytes are of mesenchymal origin (reviewed in [19]); and chromosomal disruptions in *HMGA2* lead to mesenchymal tumors, a role for the *Hmga2* gene was postulated for the normal proliferation of adipocytes.

Anand and Chada examined the role of Hmga2 in the etiology of obesity using the $Hmga2^{-/-}$ mice [12]. They showed that, in response to multiple obesity inducing stimulus, *Hmga2* expression is induced in the adipose tissue of adult wild-type mice prior to weight gain, and that $Hmga2^{-/-}$ mice are intransigent to obesity [12].

In the first set of experiments, mice were subject to the obesity-inducing stimulus of a high fat diet. As expected, after 26 weeks, wild-type mice fed the high fat diet developed obesity as compared to littermates fed the standard diet. In contrast, there was not a significant difference in weight in the *Hmga2* null mice fed either the chow or the high fat diet. Strikingly, there was also no significant difference in weight in the *Hmga2* heterozygotes fed either the chow or high fat diet, demonstrating that inactivation of a single allele of *Hmga2* is sufficient to render animals resistant to diet-induced obesity (Fig. 1, reproduced from [12]). Notably, the resistance of *Hmga2* null and *Hmga2* heterozygous mice to diet-induced obesity could not be attributed to a decrease in food intake.

$Hmga2^{-/-}$ mice were then crossed with the leptin-deficient *obese* mice, $Lep^{ob/ob}$ mice, as leptin deficiency is a far more potent obesity-inducing stimulus than a high-fat diet. The absence of Hmga2 reverted the obesity resulting from leptin deficiency as seen in the $Hmga2^{-/-}$, $Lep^{ob/ob}$ mice. Additionally, the lack of a single allele of *Hmga2* reduced obesity but did not render the mice completely resistant to weight gain. Interestingly, the $Hmga2^{-/-}$, $Lep^{ob/ob}$ mice weigh more than the $Hmga2^{-/-}$ mice. It is likely that the minor difference seen in the weight of the $Hmga2^{-/-}$ mice and the $Hmga2^{-/-}Lep^{ob/ob}$ mice is due to the presence of hypertrophic adipocytes in the $Hmga2^{-/-}$ $Lep^{ob/ob}$ mice (Fig. 2, reproduced from [12]). In the case of leptin deficiency, the increase in adipose tissue mass is due to both hypertrophy and hyperproliferation (or hyperplasia) of the fat cells [12].

Adipose tissue expansion is observed during normal development as well as in obesity, and is a result of both adipocyte hypertrophy and hyperplasia. Hypertrophy is thought to be the initial event that occurs during the development of obesity (reviewed in [20]). However, adipocytes eventually reach a maximal size beyond which normal diffusional processes cannot occur with sufficient rapidity, and thus there is a requirement for the generation of new preadipocytes in order to continue the expansion of adipose tissue mass. As shown by Anand et al., the $Hmga2^{-/-}$ mice have fat pads marked by decreased cellularity, most likely due to a deficiency in adipocyte proliferation. It is of interest that the absence of *Hmga2* by itself is sufficient to suppress the effects of leptin deficiency, and implies that peripheral targets affecting adipogenesis could be targeted by drug intervention to treat obesity.

Zhou et al. [5] have proposed a model whereby the proliferative expansion of preadipocyte populations requires Hmga2 expression (Fig. 3). Therefore, null mice have a limited capacity for the expansion of the pool of preadipocytes resulting in fewer mature adipocytes [12]. Whereas this hypothesis reflects the normal expression pattern of Hmga2 in undifferentiated mesenchymal cells in adipogenesis and obesity, this contrasts with the misexpression of Hmga2 in differentiated mesenchymal cells, as observed in lipomas and other differentiated mesenchymal tumors. Hmga2 misexpression in differentiated mesenchymal cells cases aberrant proliferation and, ultimately, a tumor. Therefore, the differentiation status of the Hmga2 expressing cell is critical in determining whether differentiation and proliferation proceeds in a regulated and orderly fashion.

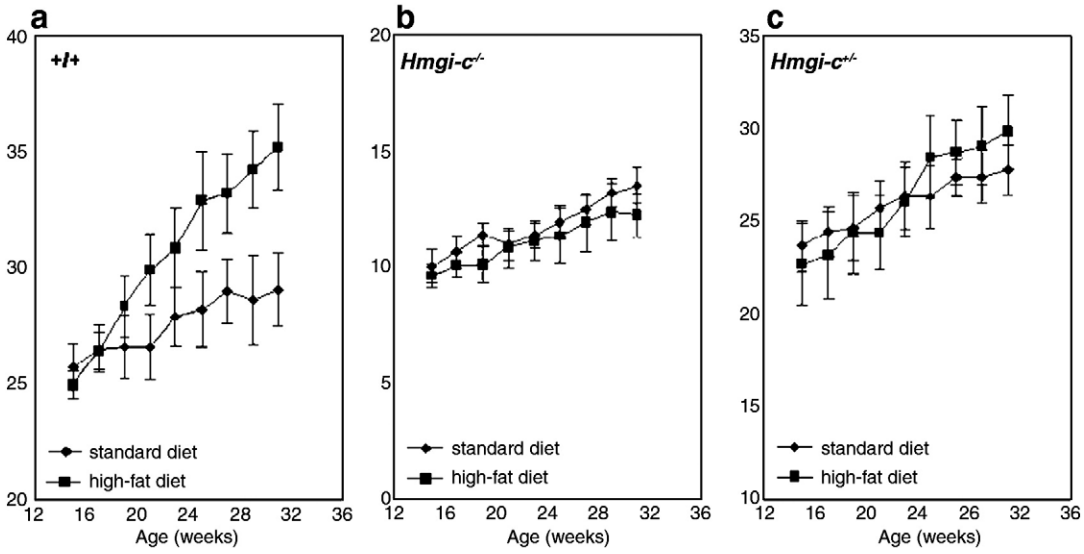

Fig. 1. $Hmga2^{-/-}$ and $Hmga2^{+/-}$ mice are resistant to diet-induced obesity. Growth curves of wild-type (a), $Hmga2^{-/--}$ (b) and $Hmga+^{-/-}$ (c) mice fed either a high-fat or a standard diet are shown. Growth measurements were initiated at 6 weeks and at least six mice were used for each growth curve. No sex-related differences were observed. The total weight gain by wild-type mice on the high fat diet was 21% (35.24 ± 1.86 g versus 29.04 ± 1.59 g, $P = 0.03$). *$P < 0.05$. Body weight in grams (g) is provided as the mean ± S.E.M. (reproduced from [12]).