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The p200 family protein p204 as a modulator of cell proliferation and differentiation: a brief survey

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Abstract

The expression of the murine p200 family protein p204 in numerous tissues can be activated by a variety of distinct, tissue-specific transcription factors. p204 modulates cell proliferation, cell cycling, and the differentiation of various tissues, including skeletal muscle myotubes, beating cardiac myocytes, osteoblasts, chondrocytes, and macrophages. This protein modulates these processes in various ways, such as by (1) blocking ribosomal RNA synthesis in the nucleolus, (2) inhibiting Ras signaling in the cytoplasm, (3) promoting the activity of particular transcription factors in the nucleus by forming complexes with them, and (4) overcoming the block of the activity of other transcription factors by inhibitor of differentiation (Id) proteins. Much remains to be learned about p204, particularly with respect to its expected involvement in the differentiation of several as yet unexplored tissues.

Keywords

Cell proliferation; Differentiation; Interferon-inducible protein; p204; p200 family

The p200 proteins

Interferons are cytokines with antimicrobial, cell proliferation inhibitory, and differentiation and immunomodulatory activities [1,2]. The findings that they induce mRNA and protein expression prompted the search for interferon-activatable genes and for the mRNAs and proteins that the respective genes encode [3,4]. The first such murine mRNA (202 mRNA) cloned by this approach was translated into the protein designated as p202 [5,6]. This turned out to be a member of a family (p200 family) of at least ten homologous proteins. These proteins are encoded by ten or more genes that evolved by repeated gene duplications and form a cluster of adjacent genes on murine chromosome 1 [6–8]. A homologous human p200 (also designated as HIN200) protein family is also interferon-inducible and also encoded by a gene cluster [9]. Most of the p200 proteins have a 5' terminal Dapin domain followed by one or two partially conserved 200 amino acid sequences (200× domains) [7,10]. The counterparts (orthologs) between the genes of the human and the murine clusters remain to be established. The murine p204 protein sequence is most similar to the human IFI16 protein sequence [6,7,11]. However,

this similarity does not prove that the two proteins are counterparts. The AIM2 protein from the human family is highly similar in sequence to one of the proteins of the murine family. The human AIM2 was discovered and named earlier [12], and because of its high similarity to human AIM2, the murine protein reported later was also called AIM2 [9].

It is puzzling that (1) the murine p200 proteins include two closely related members, p202a and p202b [13], both of which lack a 5' terminal Dapin domain, whereas (2) to date no human p200 protein lacking a 5' terminal Dapin domain has been identified [9]. Since the murine p202a and p202b proteins are highly multifunctional, one would expect that one or more human counterpart(s) lacking the Dapin domain would have been identified.

Both human and murine p200 genes exist that give rise to several protein isoforms due to multiple splicing [11,14,15]. Thus, one or more human p200 protein(s) with no Dapin domain [possibly counterpart(s) of p202a and/or p202b] may (1) be encoded (not only) by one or more as yet unidentified human gene(s), (2) but also by one or more novel protein isoform(s) encoded by one or more known gene(s), or (3) may also be formed from known or novel p200 protein(s) by appropriate modification (e.g., by cleavage).

Much research effort has been devoted to the exploration of the biological and biochemical functions of the p200 proteins [4,9,10,16]. These studies have revealed the involvement of these proteins in the modulation, among others, of cell proliferation, cell cycling, apoptosis, differentiation, malignancy, and the immune system. The p200 proteins exert their activities primarily by binding and modulating the activities of numerous transcription factors, growth regulators, oncoproteins, and tumor suppressor proteins [9,10,16–20]. Here, we briefly survey the activities of the multifunctional p200 family protein p204 (for a detailed review, see [21]) and suggest possibilities for future experiments.

Exploration of the modes of p204 action

Most of the biological research carried out in the past was initiated by first observing a biological or biochemical process and then continued by identifying the agent(s) responsible and their modes of action. In the case of p202 and p204, however, the cDNAs were first cloned and only then was a search initiated for the functions of the proteins encoded.

Both p204 and p202 inhibit the proliferation of a variety of cultured cells [22,23]. Various approaches have been used in the search for functions; these include the determination of the subcellular locations of the proteins and their tissue distribution [24–26], examination of the changes in the level of the proteins during tissue differentiation [25,26], and identification by the yeast 2-hybrid assay of the identity of the proteins binding to p204 (or p202) [17].

p204 inhibits ribosomal RNA synthesis

Part of the endogenous p204 has been found to be nucleolar in some cells [24]. This fact and the antiproliferative activity of p204 prompted us to test whether it inhibits ribosomal RNA (rRNA) synthesis that occurs in the nucleolus. It did [20]: p204 bound to UBF-1, an rRNA-specific transcription factor, thereby inhibiting its specific binding to DNA. This inhibition is likely to contribute to the antiproliferative and differentiation-promoting activities of p204.

Processes promoted by p204

Skeletal muscle myoblast differentiation

In a study previously published by our group [25] in which ten adult mouse tissues were assayed for p204, we found that the skeletal muscle had the second highest p204 level. The p204 level was strongly increased during the differentiation of cultured C2C12 myoblasts to skeletal

muscle-type myotubes, a process that involves cell fusion [25]. During this process, p204 becomes phosphorylated, and the bulk of the phosphorylated protein is then translocated from the nucleus to the cytoplasm. We observed that p204 was required for the differentiation of the myoblasts: its overexpression accelerated the process, whereas decreasing its level (by antisense RNA) inhibited it. p204, as already noted, is interferon-inducible. In our study, however, the increase in its level during myoblast differentiation was due to transcription by the muscle-specific transcription factors MyoD and myogenin [25]. p204 enabled the differentiation, at least in part, by overcoming the known inhibition of MyoD and myogenin activity by the Id (inhibitor of differentiation as well as of DNA-binding) proteins [26]. This inhibition is known to block the binding of MyoD and myogenin to DNA and thus interferes with the synthesis of numerous muscle proteins, thereby blocking the differentiation [27–29]. In our study, p204 overcame this inhibition by (1) binding to the Id proteins, (2) thereby promoting their nuclear export signal-dependent translocation from the nucleus to the cytoplasm, consequently separating the Id proteins from the transcription factors which, in the nucleus, they inhibited, and (3) accelerating their ubiquitination, and thereby their degradation in the cytoplasm by proteasomes [26,30].

Beating cardiac myocyte differentiation

In the same study on the distribution of p204 in adult mouse tissues, we found that the level of p204 was barely detectable in the heart of early mouse embryos (10.5 days), whereas in the adult heart, the level was the highest among the ten tissues tested [25]. p204 expression also increased strongly in the course of the differentiation of cultured P19 embryonal carcinoma stem cells to beating cardiac myocytes in response to dimethyl sulfoxide (DMSO) treatment [31]. Furthermore, ectopic p204 could substitute for DMSO in inducing the differentiation, whereas p204 antisense RNA blocked the process. In this study, p204 expression was synergistically transactivated by the cardiac Gata4, Nkx2.5, and Tbx5 transcription factors [31]. The Id proteins (Id1, Id2, or Id3) inhibited the differentiation of P19 cells as a result of their binding to Gata4 and Nkx2.5 and by inhibiting the binding of these transcription factors to each other and to DNA. This, in turn, blocked the activation of expression of numerous cardiac proteins [30].

Similarly to its action in skeletal muscle differentiation, in heart differentiation, p204 overcomes the inhibition by Id proteins by binding to them, promoting their translocation from the nucleus to the cytoplasm, and enhancing their degradation by ubiquitination followed by proteasomal digestion [26,30].

Osteoblast differentiation

The level of p204 increased during the bone morphogenetic protein 2 (BMP2)-induced differentiation of mesenchymal C2C12 cells to osteoblasts [32]. Furthermore, overexpression of p204 enhanced this differentiation in vitro. The induction of p204 by BMP2 is mediated by the Smad transcription factor complex. p204 is required in osteogenesis, among other reasons as a promoter of Cbfa1 transcription factor-dependent gene activation. This promotion activity involves the formation of a ternary complex in which pRb binds p204 to Cbfa1 [32,33]. The complex then binds to the promoter and transcribes, among others, the osteocalcin gene [33, 34].

Id proteins, also involved in osteogenesis, are upregulated by BMP2 [35–37]. However, Wnt, which triggers terminal differentiation of osteoblasts, also promotes a rapid decrease in Id level [38]. This decrease is required since Id proteins bind to Cbfa1, thereby inhibiting its DNA binding and transcription of the alkaline phosphatase and osteocalcin genes that are involved in the differentiation [35]. p204 overcomes the inhibition by Id proteins in osteogenesis in the same way as it does in skeletal muscle myoblast and cardiac myocyte differentiation [39]. Thus,

in osteogenesis, p204 boosts Cbfa1-dependent transcription in two ways: (1) by boosting the transcription, forming a ternary complex p204-pRb-Cbfa1, and (2) by overcoming the inhibition of Cbfa1 by Id proteins [32,39].

Chondrocyte differentiation

Chondrocyte differentiation, which is crucial for the development of long bones, involves the cessation of the proliferation of chondrocytes and their conversion to hypertrophic chondrocytes [40]. The role of p204 in this process was studied in a model system in which C3H10T1/2 cells were induced to differentiate by BMP2 [41]. BMP2 activates the expression of p204 by Cbfa1 [42]. Overexpression of p204 accelerates chondrocyte hypertrophy, whereas knockdown of the p204 level abolishes this process. In the process, p204 associates with Cbfa1 [42]. The resulting p204-Cbfa1 complex promotes the differentiation of chondrocytes by (1) decreasing the expression of parathyroid hormone/parathyroid hormone-related peptide receptor (PTHrP), an agent promoting the proliferation and delaying the differentiation of chondrocytes, and (2) stimulating the expression of Indian hedgehog protein (Ihh), an agent promoting the differentiation [42]. Chondrocyte differentiation is also regulated by p202 [43].

Macrophage differentiation

p204 is expressed in various mature monocyte and macrophage cells. Moreover, macrophage colony stimulating factor (M-CSF) or leukemia inhibitory factor (LIF) has been found to induce p204 expression as well as the differentiation to macrophages of the myeloid progenitor line FD-Fms. Constitutive expression of p204 strongly decreased the M-CSF (and interleukin3) dependent proliferation of the FD-Fms cell line, whereas it promoted its M-CSF-induced differentiation to macrophages [44]. The 204 mRNA level is lower in less mature (CD4+ CD8+ double positive) thymocytes than in more mature (CD4+ or CD8+ single positive) thymocytes, which may indicate a role for p204 in lymphocytic differentiation [45].

p204 as a negative feedback inhibitor of Ras activity

Many of the effects of p204 (e.g., the modulation of transcription factor activity) occur in the nucleus. However, as noted, in various processes of differentiation, part of p204 is translocated to and accumulates in the cytoplasm [25,30,31]. A yeast two-hybrid assay revealed that cytoplasmic proteins binding p204 include the multi-functional H and K-Ras signaling proteins [17]. This binding was verified by pull-down assays with purified proteins. These assays also showed that p204 binds to activated H-RasGTP (but not to inactive H-RasGDP) as well as to both the wild-type Ras proteins and mutated Ras oncoproteins [17] (that are the most frequently activated oncoproteins in cancer) [46,47]. p204 inhibits (1) the cleavage of RasGTP to RasGDP by RasGAP, (2) the binding to RasGTP of Raf, PI3K, and Ral-GDS, effectors of Ras signaling and, thereby, (3) the promotion by the Ras pathway of the phosphorylation and activation of its downstream targets [17].

Ras oncoprotein promotes the phosphorylation and translocation of p204 from the nucleus to the cytoplasm, thereby enabling the interaction between p204 and Ras [17]. Furthermore, the Ras oncoprotein or activated wild-type Ras induces the expression of Egr-1, a transcription factor activating p204 expression. A single copy of K-ras oncogene in a murine embryo cell was found to induce a high level of p204 as well as the translocation of a portion of p204 to the cytoplasm. Thus, p204 can serve as a negative feedback inhibitor of Ras, which may contribute to its antitumorigenic effect [17].

p204 promotes the replication of murine cytomegalovirus

Although p204 can be induced by interferons, which (besides having other activities) are important antiviral agents, there have been no reports of p204 inhibiting the replication of any virus. Remarkably, however, p204 has been found to promote the replication of cytomegalovirus (CMV) in embryo fibroblasts, and the infection of these cells by CMV strongly boosted p204 expression transcriptionally [48,49].

Induction of p202 and p204

The p200 family proteins are inducible by interferons and were initially discovered in a search for interferon-inducible proteins [5,6]. The extent to which interferons can induce proteins, such as the p202 proteins, can differ greatly among different strains of mice and also among cell lines derived from these [50,51]. This variation in p202 inducibility by interferons may be due to possible differences in the activity of some of the transcription factors among these strains [52] and/or to the reported polymorphism (i.e., sequence differences) among the various mouse strains in the 5' regulatory region of the Ifi202a and/or the Ifi202b genes [53]. As described in the sections devoted to the promotion by p204 of the differentiation of muscle myoblasts, cardiac myocytes, osteoblasts, and chondrocytes, the expression of p202 and p204 is promoted in numerous tissues by distinct tissue-specific transcription factors (whose activities are not dependent on interferon) [25,31,32,42,54]. This fact is the basis of the finding that both the amounts and the distribution of p204 (also of p202) among the five tissues examined from adult mice were very similar in wild-type mice and in mice not responsive to interferon (due to lacking interferon receptors) [13]. Thus, at least in "healthy" mice, the bulk of p204 and p202 is not formed in response to interferon action.

Further exploration of p204 action

Further exploration of the functions of p204 would be greatly facilitated by the generation of mice in which p204 formation could be inhibited by inducible tissue-specific and/or embryo developmental stage-specific knockout [55]. The availability of such mice would open the door to new research possibilities with p204, including the testing of conclusions drawn from experiments with cultured cells.

The adult mouse myocardium whose myocytes do not proliferate has a remarkably high level of p204 and a very low level of Id proteins [26,30]. What we have learned about the proliferation boosting and differentiation inhibitory activities of the Id proteins [26–30] and about the proliferation inhibitory and differentiation boosting activities of p204 [20,21,26,30,39] may serve as the basis for an intriguing exploration involving myocytes. Such an exploration may consist of testing whether a decrease in the p204 level, as well as an increase in the Id protein level (together with other changes if required), in a culture of beating, terminally differentiated (and nonproliferating) myocytes could induce this culture to dedifferentiate (possibly transiently) to the extent of allowing some cell proliferation. If so, one could attempt to trigger the redifferentiation of the proliferated cells. A positive outcome may provide the basis for intriguing in vivo explorations.

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