INTERFERON-INDUCIBLE P202 IN THE SUSCEPTIBILITY TO SYSTEMIC LUPUS

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1. ABSTRACT

Systemic lupus erythematosus (SLE) is the prototype systemic autoimmune disease, which has potential to involve multiple organ systems. Studies in human SLE patients and murine models of lupus have indicated that genetic predisposition plays a crucial role in the development of this disease. To identify the genetic basis of human lupus and to understand the molecular mechanisms, mouse models of SLE have been studied. Generation of mice congenic for the Nba2 locus on the C57BL/6 genetic background, coupled with gene expression profiling, recently identified the interferon-activatable Ifi202 gene (encodes the protein p202) as a candidate lupus-susceptibility gene. The protein p202 is a member of the 200-protein family. The family includes structurally and functionally related mouse and human interferon-inducible proteins. The protein p202 (52-kDa) is a relatively well-characterized phosphoprotein in the family with demonstrated ability to control cell-signaling pathways regulating cell proliferation, survival, and differentiation. Here, we review what is known about the gene Ifi202 and the protein it encodes. Moreover, we discuss how an understanding of the role of p202 in cell growth regulation, particularly in cells of the immune system, will help elucidate the molecular mechanisms contributing to the development of lupus.

2. INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disorder that predominantly affects women of childbearing age (1-5). The disease is a heterogeneous syndrome with a complex immunopathogenesis (3, 4). Clinically, many major organ systems, including the skin, kidneys, and nervous system can be affected (3, 4). Based on genetic linkage studies, there is considerable evidence that SLE is polygenic disease, and multiple chromosomal loci appear to be involved in the development of the disease (1, 6, 7). Despite the polygenic nature of the disease and
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![Image](72x512 to 288x720)

**Figure 1.** Schematic structural representation of the 200-family proteins.

considerable genetic heterogeneity, a common denominator among SLE patients is the production of IgG autoantibodies, particularly to nuclear antigens (1-5).

In order to identify SLE susceptibility genes and to elucidate the molecular mechanisms by which the products of these genes contribute to SLE, murine models of human SLE have been studied (7-12). Studies of murine models of lupus have provided important insight into the immunopathogenesis of IgG autoantibody production and lupus nephritis (11, 12). Strikingly, genetic studies using these mouse models have revealed that, like in human SLE, several chromosomal loci contribute to the disease in mouse lupus (7, 11, 12). Furthermore, based on several genetic studies, it is apparent that some chromosomal regions consistently show relatively strong linkage with the development of lupus (11). Especially, the Nba2 locus on distal chromosome 1 has emerged as an important basis for a role of p202 in the susceptibility to lupus.

Mice congenic for the Nba2 locus (on the C57BL/6 genetic background; indicated as B6.Nba2) showed elevated serum autoantibodies characteristic of lupus and an increase in splenic B cells at an early age (15). Studies also showed that these mice had increased levels of interferon-inducible p202 in their splenic B cells, which correlated with B cell accumulation, apoptosis-resistance, and lupus susceptibility. These studies, thus, identify the IFN-activatable Iifi202 gene as a candidate lupus-susceptibility gene in mice (15).

Interferons (alpha, beta, and gamma) are a family of cytokines with multiple biological activities, including inhibition of viral replication, cell growth inhibition and immunomodulation (16, 17). Consequently, interferons (IFNs) are used in the clinic to treat several human diseases, including some cancers (16, 18), hepatitis C virus infection and condition-specific autoimmune disorders, such as multiple sclerosis (19). Interestingly, therapy with IFN-alpha is known to be associated with the development of lupus-like autoreactive antibodies (20) and, indeed, increased levels of IFN-alpha are detected in sera of SLE patients (20, 21). Consistent with a role of IFNs in the development of lupus disease, deletion of the IFN-gamma receptor (22) or depletion of IFN-gamma in lupus-prone (NZB x NZW)F1 mice (23) prevents autoantibody production and glomerulonephritis. Because IFN-inducible proteins mediate the biological activities of IFNs (24), together, these observations provide support for the idea that the IFN-inducible proteins play an important role in the development of lupus disease.

The IFN-activatable gene Iifi202 encodes the protein p202, which has demonstrated ability to regulate cell proliferation, survival, and differentiation (25). Here, we discuss what is known about the gene Iifi202 and the protein it encodes, regulation of its expression, and the basis for a role of p202 in the susceptibility to lupus.

3. THE GENE IIFI202 IS A MEMBER OF THE 200-GENE FAMILY

The gene Iifi202 is a member of the 200-gene family (26-29). The family includes structurally related mouse genes (Iifi202a, Iifi202b, Iifi203, Iifi204, and D3) that form a tight cluster of linked genes on the distal part of mouse chromosome 1 (30-33). It is likely that the gene family arose from a common ancestor gene by repeated duplication (34). Importantly, this region of mouse chromosome 1 (~ 6,000 kb) is syntenic to a region at 1q22-23 on human chromosome 1 (35), which contains three highly homologous genes of the family: IFI16, myeloid nuclear differentiation antigen (MNDa), and AIM2 (absent in gelanoma-2) (26, 29). The gene IFI16 is a human homologue of mouse Iifi202 gene (29). Genetic alterations in the 1q region have been reported in several human diseases, including cancers (36), and susceptibility loci for SLE have been mapped to this region (7, 13).

The proteins in the 200-family share at least one unique repeat of partially conserved 200-amino acids (either a type or b type) (26, 29) (see figure 1). The 200-amino acid repeat may have role in modulating the transcription of growth-regulatory genes either directly or indirectly (25, 29) (see below). The longest continuous sequence, which is conserved among the 200-family proteins is a short motif M(F/L)HATVA(T/S) (26). The structural and functional significance of this motif in the 200-family proteins remains to be established. Furthermore, the proteins (except p202a and p202b) in the family, including the protein encoded by IFI16 gene, also share a newly identified protein domain CARD/DAPIN/PYRIN in their N-terminus (37-39) (see figure 1). The domain is thought to be involved in protein-protein interactions and is found in several other key proteins known to participate in the regulation of apoptosis and inflammation (37, 38).
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Although the role of the 200-family proteins in IFN action remains to be established, there are indications that these proteins participate in the regulation of cell proliferation, survival, and differentiation (25, 29). Consistent with this hypothesis, coding region frame-shift mutations have been found in the Ifi202 gene in human colorectal cancers, resulting in a truncated non-functional protein (40). This observation raises the possibility that the functional inactivation of the 200-family proteins could provide a growth advantage to certain type of cells.

4. THE REGULATION OF p202 EXPRESSION

Screening of adult mouse tissues and organs for expressed levels of 202-specific mRNA revealed that basal levels of the 202 RNA are detectable in a variety of adult tissues (derived from mice of mixed 129sv and BALB/c genetic background), including the ovary, thymus, spleen, skeletal muscles, liver, and heart (33). The levels of the 202 RNA are relatively low in the brain, kidney, lung, and testicles. Consistent with the above observations, C57BL/6 and B6.Nba2 mice (congenic for the Nba2 locus) had undetectable levels of the 202-specific mRNA in their kidneys (15). However, it remains to be seen whether the expression of the 202 RNA is detectable in other tissues of the congeneric mice.

Interestingly, analysis of Ifi202 gene expression in spleen cell subsets (derived from the NZB strain of mice before autoantibody production) by quantitative PCR revealed that Ifi202 RNA was highly expressed in B220" B cells and non-B/non-T cells, compared to CD4" T cells (15). These data suggest that the increased expression of p202 in spleens of these mice is primarily due to increased expression in a subset of B-lymphocytes and not CD4" T-lymphocytes.

4.1. The expression of p202 depends on the genetic background of mice.

The IFN responsiveness of the Ifi202 gene is dependent on the mouse strain. For example, injection of a potent IFN inducer, the synthetic dsRNA poly rI: rC, increased 202 RNA (probably both 202a and the 202b) expression in DBA/2, BALB/c, and C3H/HeJ mice, but not in C57BL/6 mice and cell lines derived from this strain (41). This lack of Ifi202-gene inducibility in C57BL/6 mice was selective, as a different interferon-inducible gene (2'-5'-oligoadenylate synthetase) was equally induced in all four strains of mice tested.

Consistent with the above observations, we recently reported that in mouse whole spleen samples (which includes B, T, non-B, and non-T cells), basal levels of the 202-specific RNA as well as protein depend on the genetic background of the mice (15). Hence, cells from C57BL/6 and NZW inbred strains of mice expressed undetectable levels of p202 whereas cells from the NZB strain expressed high levels of 202 RNA. Notably, B6.Nba2 mice (congenic for the NZB-derived Nba2 locus) expressed 202 RNA at levels comparable with the NZB strain. Moreover, increased levels of p202 were detectable in samples derived from NZB and B6.Nba2 strains of mice (15). It is important to note that in extracts derived from the NZB strain of mice, additional protein bands not present in other mouse strains were detected. The identity of these additional protein bands detected by the antiserum to p202 remains to be established.

Comparison of the 5'-regulatory region of the Ifi202-gene among various strains of mice revealed several sequence polymorphisms (15). The presence of a single nucleotide polymorphism (C-> T at position +95) was used as a marker for the haplotype of sequence differences. This single nucleotide polymorphism (SNP) strongly correlated with lack of p202 expression in some strains of mice, such as C57BL/6 and NZW. It is important to point out that this SNP does not fall within the two known IFN-responsive cis-elements (ISRE-like sequence and Friedman-Stark sequence) present in the 5'-regulatory region of the 202-gene (34). Although it remains to be seen which of the 5'-regulatory region polymorphisms in NZB versus B6 mice contributes to the differential expression of p202, there are indications that mouse strain-specific transcription factor(s) may regulate the transcription of the Ifi202-gene (42, 43). Therefore, identification of mouse strain-specific transcription factor(s) will be important to elucidate the molecular mechanisms contributing to differential regulation of p202 expression in various strains of mice, including the congeneric strain.

4.2. Transcriptional as well as post-transcriptional regulation

Basal levels of p202 can be detected in cultured fibroblasts and epithelial cells (25). It has been shown that IFN (alpha or beta) treatment of cultured fibroblasts strongly increases the levels of p202, and the increase, in part, is because of an increase in the transcriptional rate of the Ifi202 gene (44, 45). However, there is evidence that the levels of p202 are also regulated at post-transcriptional levels (33, 46). Therefore, it remains to be seen whether increases in the steady-state level of p202 in the B6.Nba2 congeneric strain of mice are entirely due to the transcriptional differences described above.

4.3. IFN-independent regulation

There is increasing evidence that the basal expression levels of p202 can be regulated by mechanisms independent of IFN signaling (47-50). Firstly, p202 is constitutively expressed in C57C12 mouse skeletal muscle cells, and the levels of the 202 RNA and protein are greatly increased during the differentiation of C57C12 myoblasts to myotubes (47). Secondly, the levels of 202 RNA and protein increase in mouse fibroblasts during their incubation under reduced serum conditions (48). Moreover, the increase in levels of p202 expression correlates with an increase in the transcriptional activity of the JunD/AP-1 transcription factor and its binding to an AP-1-like sequence present in the 5'-regulatory region of the Ifi202 gene (48). Thirdly, Notch-1 signaling has been shown to positively regulate the expression of the 202 RNA in CD4+ and CD8+ double-positive thymocytes (49). Lastly, the levels of the 202 RNA and protein decrease after increases in the levels of wild type, but not mutant, p53 (50). The decrease in levels of the 202 RNA and protein was also
observed after exposure of cells to low doses of ultra-violet light, which correlated with increases in the levels of p53.

Although, it remains to be seen whether increases in the basal levels of p202 in the congenic strain of mice are independent of IFN production, together, these observations provide support for the idea that p202 levels are also regulated independent of IFN signaling.

5. THE PROTEIN p202a

The protein p202a (hereafter referred as p202) is the first and best-characterized member of the 200-protein family (25, 34, 51). It is a phosphoprotein in mouse fibroblasts and detected as a 52-kDa protein in the nucleus (51, 52). IFN-induced increases in the levels of p202 in cultured mouse fibroblasts are detected both in the cytoplasm and in the nucleus (51). The cytoplasmic localization of p202 in fibroblasts is consistent with lack of a classical nuclear localization signal (NLS) in the p202 sequence (25). Therefore, it is likely that the nuclear localization of p202 is regulated and depends on factors, such as cell type. Consistent with this idea, it has been demonstrated that the large increases in the levels of p202 during differentiation of C2C12 myoblasts in vitro are associated with translocation of p202 into the cytoplasm (53).

In cultured proliferating fibroblasts (treated with IFN), p202 has relatively long half-life (>9 h) following treatment with cycloheximide, an inhibitor of protein synthesis (51). It remains to be seen whether the half-life of p202 is different in untreated fibroblasts and in lymphocytes.

The protein, p202b, which differs from p202 by only 7 amino acids out of 445, is co-expressed with p202 in several mouse tissues (33). In embryonic fibroblasts from mice in which the \( Ifi202a \) gene was knocked out, the levels of p202b are increased (33). The compensatory increase in \( Ifi202a \) gene expression probably accounts for the lack of a phenotype in the \( Ifi202a \) gene knockout mice.

Presently, it is not known whether spleen cells of B6.Nba2 congenic mice over-express p202a, p202b or both proteins. Moreover, it remains to be seen whether p202 functions are differentially regulated by the strain and/or by cell type-specific post-translational modifications, such as phosphorylation.

6. ROLE OF p202 IN CELL GROWTH REGULATION

Regulation of cell cycle plays an important role in lymphocyte differentiation, apoptosis, effector function, memory acquisition, and tolerance induction (54, 55). In this regard, genetic deficiency in the levels of cell cycle regulators, such as the cyclin-dependent kinase inhibitor (CDKI) p21\(^{\text{WAF1}} \) (56) and the transcription factor E2F2 (57), in immune cells has been shown to contribute to the development of SLE phenotype in mice. Furthermore, apoptosis has been implicated in lupus pathogenesis (58). Thus, the demonstrated ability of p202 to control cell cycle, survival, and differentiation may provide the basis for its role in the susceptibility to lupus (see figure 2 and below).

Ectopic expression of p202 in cultured cells (both normal and cancer cells) results in inhibition of: (1) colony formation (51, 59-61); (2) anchorage-independent growth of transformed cells (62), and (3) tumor formation in nude mice (63). Additionally, using ectopic and orthotopic xenograft models, it has been demonstrated that p202 expression is associated with multiple antitumor activities that include inhibition of tumor cell growth, reduced tumorigenicity, prolonged survival, and remarkably, suppression of metastasis and angiogenesis (64). Thus, these results provide support for the idea that p202 contributes to the anti-proliferative and antitumor activity of IFNs.

Surprisingly, decreases in the levels of p202 in mouse fibroblasts also result in a decreased cell proliferation rate without detectable apoptosis (65). Thus, ectopic over- or under-expression of p202 in a variety of cycling cells (under normal growth conditions) results in reduced cell growth rate without apoptosis. Together, these observations suggest that p202 is a potent modulator of cell growth and appropriate levels of p202 are important for normal cell cycle progression.

6.1. How does p202 retard cell proliferation?

Inducible expression of p202 in cultured mouse fibroblasts at physiological levels results in increases in the steady-state levels of CDKI p21\(^{\text{WAF1}} \) RNA and protein (independent of p53) and hypophosphorylation of Rb (66). These data suggest that p202 mediates the anti-proliferative effect of IFNs, in part, by inhibiting the activity of the cyclin-dependent kinases (CDKs) that are needed for normal cell cycle progression. Importantly, by reducing the inhibitory phosphorylation of Rb (and perhaps other pocket proteins) by CDKs, p202 may potentiate E2F-mediated transcriptional repression of the E2F responsive genes (67). Consequently, depending on the cell system, cell growth arrest or cell growth retardation results. Because p21\(^{\text{WAF1}} \) could also inhibit the transcriptional activity of E2F independent of Rb (68), p202-mediated increases in the levels of p21\(^{\text{WAF1}} \) could also inhibit E2F activity independent of Rb (and other pocket proteins).
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Figure 3. p202 and the Rb/E2F pathway: potential functional interactions.

The amino acid sequence of p202 contains the Rb binding motif LxCxE (69), and p202 binds to Rb and other pocket proteins (p107 and p130) in vitro and in vivo (61, 69). Moreover, a point mutation in the pocket domain of Rb (mutation 706) that renders Rb functionally inactive in human cancers, resulted in significantly reduced binding of Rb to p202 in vitro (69). This observation indicated that the ‘pocket’ domain in Rb is a main binding site for p202. Interestingly, p202 also binds to Rb in the N-terminus (amino acids 1-255) (69). Consistent with the binding of p202 to Rb, the expression of p202 was shown to inhibit colony formation in an Rb-dependent manner (70).

Constitutive over-expression of p202 in the human tumor cell line DU-145, which does not harbor functional Rb, results in inhibition of colony formation (62). In contrast, over-expression of p202 in the Saos-2 cell line, which harbors a mutant Rb, does not result in inhibition of colony formation (Choubey, unpublished data). In addition, co-expression of p202 with Rb in Saos-2 cells results in significant inhibition of colony formation. These latter observations provide support for the idea that p202 and Rb cooperate to inhibit cell growth. Although, p202-mediated inhibition of colony formation depends on Rb (and possibly other pocket proteins) in some cell systems, other mechanisms, such as through induction of p21WAP, are likely to be operative in certain types of cells.

Together, the above studies provide support for the idea that p202 negatively regulates cell cycle progression and potentiates differentiation of certain cell types, in part, through the Rb/E2F pathway (see figure 3 and below). Consistent with this idea, over-expression of E2F4 partially relieves p202-mediated inhibition of colony formation in mouse fibroblasts (61). In addition, the adenovirus-encoded E1A protein, which promotes cell proliferation by inactivating the Rb/E2F pathway, targets p202 for functional inactivation: the expression of E1A protein alleviates p202-mediated inhibition of cell growth and the transcriptional activity of E2F1 (71).

We recently noted that the functional inactivation of Rb by serum growth factors or by expression of viral oncoproteins, such as SV40 large T antigen, results in decreases in the levels of p202 (48). This observation, along with our previous observation that the expression of p202 functionally activates Rb (66, 69), provides support for the hypothesis that Rb and p202 are part of a positive feedback loop, which may be important for the regulation of cell growth.

It is important to note that the ability of p202 to inhibit the transcriptional activity of other factors, such as AP-1 (60) and c-Myc (72), which promote cell proliferation, makes it conceivable that p202 negatively regulates cell cycle progression in some cell systems, in part, by mechanisms independent of the Rb/E2F pathway.

6.2. CONTROL OF CELL SURVIVAL

Over-expression of p202 delays c-myc-p53-induced apoptosis in mouse Vm10 fibroblasts (50), c-myc-induced apoptosis in Rat-1 cells (under reduced serum conditions) (72), and the adenovirus-encoded E1A-mediated apoptosis of mouse Pam212 keratinocytes (under reduced serum conditions) (71). Also, in AKR-2B fibroblasts, a decrease in the basal levels of p202, under reduced serum conditions, increases susceptibility to apoptosis (65). In contrast to these studies, ectopic expression of p202 in a human breast cancer cell line (MCF-7) sensitizes cells to TNF-alpha induced apoptosis and this correlates with the inhibition of the activity of NF-kappaB by p202 (63). Jointly, these observations support the notion that IFN-inducible p202 plays an important role in the modulation of apoptosis.

Production of autoreactive antibodies is partially prevented through elimination of B cells recognizing self-antigen at various stages during development in the bone marrow and in peripheral lymphoid organs (73). B cell receptor (BCR)-mediated programmed cell death in germinal centers can be triggered by antigen binding in the absence of an interaction with CD40 ligand-bearing CD4+ Th cells. The abrogation of the apoptotic cell death in B cells after CD40 ligation is thought to be through the induction of survival factors. Interestingly, IFN treatment of a germinal center B cell line inhibits apoptosis induced by cross-linking of its antigen receptor (74). Thus, IFN-inducible proteins may provide survival signals to these cells. Consistent with this idea, increased expression of p202 (about 10-fold more) in B cells derived from B6.Nba2 congenic mice, compared to C57BL/6 control mice, correlated with inhibition of apoptosis following ligation with anti-IgM (15). Cell death by apoptosis in immature and naïve B cells is important for maintenance of B cell tolerance (73). Consequently, the increased expression of p202 in B6.Nba2 and NZB B cells may inhibit clonal deletion of B cells, and contribute to the development of murine lupus.

Remarkably, congenic B6.Nba2 mice (but not control mice) were noted to have splenomegaly even at an early age (15). Furthermore, splenomegaly was associated
The ability of p53 to act as a tumor suppressor depends on its ability to act as a transcription factor (81). Activation of p53 results in transcriptional activation of its target genes, which include p21\(^{WAF1}\) and mouse double minute-2 (mdm2) (81). It has been demonstrated that CDKI p21\(^{WAF1}\) is an important mediator of p53-mediated cell growth arrest (82). However, several p53-regulated genes, encoding proapoptotic proteins, appear to contribute to p53-mediated apoptosis (82).

The ability of p202 to inhibit the transcriptional activity of p53 in transfected fibroblasts makes it conceivable that p202 influences p53-mediated cell growth arrest and apoptosis (83). However, the ability of p202 to increase the levels of p21\(^{WAF1}\) in some cell systems (66) provides support for the hypothesis that p202 allows cell growth arrest after induction of p53. Moreover, the ability of p202 to delay p53-mediated apoptosis in several well-characterized cell systems (50, 71, 72) provides support for the idea that p202 influences p53-induced apoptosis. In this regard, it will be important to determine whether increases in the levels of p202 in B cells, derived from lupus-prone strains of mice, increase cell survival by inhibiting p53-mediated transcription.

Our recent observations have also revealed that the Ifi202-gene is a target of p53 for transcriptional repression (50). These observations, along with our earlier observation that p202 inhibits the transcriptional activity of p53 in 10T1/2 fibroblasts (83), suggest that p202 and p53 are part of a negative feedback loop (see figure 4). This loop may be important in modulating apoptosis in some physiological settings. Defects in the regulation of p202 levels by p53 may contribute to increased susceptibility to autoimmune diseases, such as systemic lupus, and spontaneous tumors of the immune cells. Consistent with this hypothesis, mice deficient in p53 (p53\(^{-/-}\)) are susceptible to lymphomas involving the thymus and other visceral organs, including the spleen (84).

### 6.3. Role of p202 in differentiation

The levels of 202 RNA are relatively high in mouse skeletal muscles (47). In addition, the levels of 202 RNA and protein increase (more than 10-fold) in mouse C5\(\text{C}_{12}\) myoblasts upon incubation in differentiation medium (reduced serum) (47). These observations advance the interesting possibility that p202 may have a role in the differentiation of these cells. Consistent with this idea, ectopic expression of p202 before differentiation of C5\(\text{C}_{12}\) myoblasts inhibited differentiation and correlated with an inhibition of the transcriptional activity of MyoD and myogenin (47). While these observations suggest that the timing of increases in the levels of p202 is important for proper differentiation of these cells, these studies also raise the possibility that p202, like other proteins such as HBP1, is a part of a differentiation checkpoint (85). In this case, p202 may augment differentiation of myoblasts by promoting the cell cycle exit and inhibiting differentiation-related apoptosis (by increasing the levels of CDKI p21\(^{WAF1}\)).

Additionally, it is important to point out that the regulation of the 202 RNA in CD4\(^+\) and CD8\(^+\) double-
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7. TRANSCRIPTIONAL MODULATION BY p202

The ability of p202 to control cell proliferation, survival, and differentiation might be correlated with its ability to inhibit the transcriptional activity of several factors (25). These factors include: c-Fos (60), c-Jun (60), c-Myc (72), MyoD (47), myogenin (47), NF-kappaB (p50 and p65) (60), and E2Fs (E2F1 and E2F4) (59, 61). In most of these cases, the binding of p202 to these factors results in inhibition of the sequence-specific DNA binding activity (see table 1).

<table>
<thead>
<tr>
<th>Transcription factor</th>
<th>Potential mechanism(s)</th>
<th>Gene(s) tested</th>
<th>Reference</th>
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<tbody>
<tr>
<td>E2F4</td>
<td>inhibition of DNA-binding</td>
<td>reporter gene, PCNA, and b-myb genes</td>
<td>59</td>
</tr>
<tr>
<td>AP-1 (c-Fos and c-Jun)</td>
<td>inhibition of DNA-binding</td>
<td>reporter gene and collagenase gene</td>
<td>60</td>
</tr>
<tr>
<td>p53</td>
<td>not known</td>
<td>reporter genes</td>
<td>83</td>
</tr>
<tr>
<td>NF-kappaB (p50 and p65)</td>
<td>inhibition of DNA-binding</td>
<td>reporter gene and interferon-beta gene</td>
<td>60</td>
</tr>
<tr>
<td>c-Myc/Max</td>
<td>inhibition of dimerization</td>
<td>reporter gene, ornithin decarboxylase and alpha-prothymosin genes</td>
<td>72</td>
</tr>
<tr>
<td>MyoD</td>
<td>inhibition of expression and DNA-binding</td>
<td>reporter gene</td>
<td>47</td>
</tr>
<tr>
<td>Myogenin</td>
<td>inhibition of DNA-binding</td>
<td>reporter gene</td>
<td>47</td>
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7.1. Regulation of the E2F family of transcription factors

The E2F family consists of six E2Fs that heterodimerize with one of two different proteins, first identified as differentiation proteins (DP), to create 12 protein homodimers that have little or no affinity for DNA (86). The E2Fs can be divided into three subgroups: (i) E2F1, E2F2, E2F3, which are highly related and display overall maximal expression in G1 to early S phase of the cell cycle; (ii) E2F4 and E2F5, which are less responsive to changes in proliferation and lack an N-terminal domain present in E2Fs 1 to 3; and (iii) E2F6, a recently cloned E2F family member that lacks both the N-terminal region common to E2Fs 1 to 3 and the C-terminal trans-activation domain common to E2Fs 1 to 5. The E2F trans-activation domain is encoded within an acidic carboxy-terminal region that also carries a site for binding by pocket proteins (86).

Known E2F target genes are numerous and include critical cell cycle regulators (e.g., cyclins, Cdk, and Cdk inhibitors), as well as important mediators of DNA synthesis (e.g., DNA polymerase-alpha, dihydrofolate reductase, and thymidine kinase) (67). Genes controlled by E2F show low promoter activity in quiescent and early G1 phase cells and high promoter activity in late G1 and S phase cells (67, 86).

The activity of E2F2 is needed for suppression of T cell proliferation and immunologic self-tolerance (57). Consequently, mice null for E2F2 develop late-onset autoimmune features, characterized by widespread inflammatory infiltrates, glomerular immune-complex deposition, and antinuclear antibodies (57). Importantly, E2F2 appears to repress the transcription of the E2F1 whose activity is required for normal S phase entry (57). On the contrary, E2F1 positively regulates the expression of E2F2. p202 inhibits the transcriptional activity of a subset of E2F family members (59, 61). Therefore, it is possible that alterations in the levels of p202 in a subset of T cells could affect T cell proliferation and function through E2F2 levels and/or activity.

7.2. Functional interactions between p53 and p202

The expression of p202 (or IFN treatment of cells) inhibits p53-mediated transcription, whereas decreases in the levels of p202 significantly stimulate p53-mediated transcription (83). Of note, the expression of p202 does not result in inhibition of the specific DNA binding activity of p53 in gel-mobility shift assays (52). Furthermore, p202 over-expression in 10T1/2 cells results in a modest increase in the steady-state levels of p53 (83). Together, these observations indicate that in the cell systems tested so far, p202 negatively regulates the transcriptional activity of p53 without inhibiting its specific DNA binding activity and decreasing its levels. The observation that a segment of 53BP-1 (a p53 binding...
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Figure 5. Hypothetical scheme for immunopathogenic process in the development of SLE and role of p202 in survival of auto-reactive B cells.

protein shown to stimulate p53-mediated transcription in promoter-reporter assays) binds to p202 and over-expression of this segment of 53BP-1 alleviates p202-mediated inhibition of p53-mediated transcription (83), raises the possibility that p202 inhibits p53-mediated transcription by binding and sequestering 53BP-1.

As stated above, the ability of p35 to negatively regulate the expression of p202 may be important for the regulation of cell survival in immune cells (see Fig. 4).

8. FUTURE PERSPECTIVES

The past few years have provided some new appreciation for the role of the 200-family proteins in IFN action. Studies have indicated that the 200-family proteins participate in the regulation of cell growth (26, 29). The protein p202 continues to be a prototype for the 200-family of proteins.

The demonstrated ability of p202 to control cell proliferation, survival and differentiation in a variety of cell systems provides support for the idea that p202 is clearly an important mediator of the biological activities of IFNs. It will be important to determine which IFN-responsive transcription factor(s) activate the transcription of the \( \text{IFI}202 \) gene and whether additional mouse strain and/or cell type-specific transcription factors modulate the expression of the \( \text{IFI}202 \) gene. Additionally, it will be necessary to determine how post-translational modifications of p202, such as phosphorylation, and nuclear localization is regulated. Our understanding of the molecular mechanisms by which the levels of p202 and its functions are regulated is important to elucidate its role in cell growth regulation.

Several recent observations have suggested that the levels of p202 are also regulated independent of IFN signaling (25, 47-50). Therefore, it will be important to elucidate these molecular mechanisms controlling p202 expression as well. A clear understanding of all aspects of \( \text{IFI}202 \) gene expression is important to identify the physiological role of p202.

The identification of \( \text{IFI}202 \) as a candidate susceptibility gene for lupus adds an additional reason and need to understand the molecular mechanism(s) by which p202 expression is controlled and how p202 affects immune response (see figure 5). It will be important to determine why p202 expression is constitutively increased in certain lupus prone strains of mice, including the B6.Nba2 congenic strain, and how the expression differences affect cells of the immune system. In this regard, it remains to be determined which polymorphisms in the 5'-regulatory region of the \( \text{IFI}202 \) gene contribute to differential regulation of the \( \text{IFI}202 \) gene and whether mouse strain, sex, and cell type-specific transcription factors contribute to regulation of \( \text{IFI}202 \) gene expression. Moreover, it will be necessary to investigate how expression of p202 affects the biology of immune cells (B and T lymphocytes) to understand their relative contribution to the lupus phenotype (production of IgG antinuclear antibodies). Importantly, it will be necessary to identify the signaling pathway(s) and molecular mechanism(s) by which p202 controls cell survival in cells, particularly in B-lymphocytes.

Our clear understanding of the role and mechanisms of p202 action in murine models of lupus disease will provide novel insights into the signaling pathways and the molecular mechanisms contributing to the development of lupus. Furthermore, these studies will also serve as a molecular basis to study the possible role of IFN-inducible IFI16 protein (the human homologue of p202) in the signaling pathways regulating survival of B-cells in human SLE. Consequently, these studies are likely to advance our knowledge of lupus significantly, which will be helpful in considering new strategies for the diagnosis and treatment of this disease.

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10. REFERENCES

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