[Frontiers in Bioscience 7, e252-262, May 1, 2002]

INTERFERON-INDUCIBLE P202 IN THE SUSCEPTIBILITY TO SYSTEMIC LUPUS

Divaker Choubey [1] and Brian L. Kotzin [2]

[1] Department of Radiation Oncology, Stritch School of Medicine, Loyola University Medical Center & Hines VA Hospital, 2160 South First Avenue, Maywood, IL 60153, [2] Department of Medicine and Immunology, University of Colorado Health Sciences Center, Division of Clinical Immunology, 4200 East Ninth Avenue, Denver, CO 80262

TABLE OF CONTENTS

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

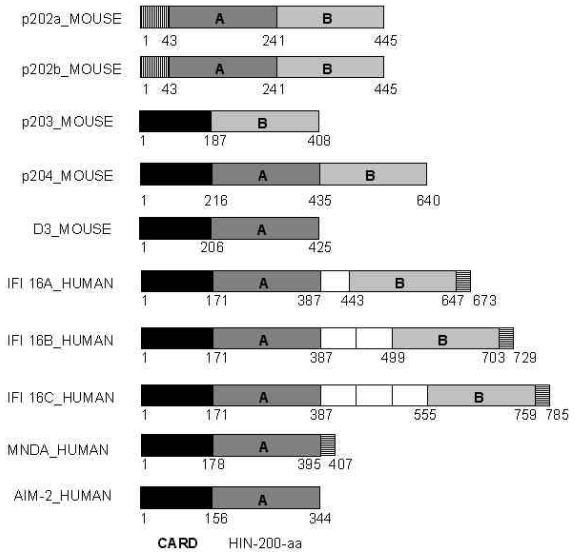

Figure 1. Schematic structural representation of the 200-family proteins.

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 mouse lupus (11). Especially, the *Nba2* locus on distal chromosome 1 has emerged as an important contribution to lupus susceptibility in the New Zealand hybrid model of lupus (9-14). The locus is linked to the development of lupus nephritis, production of multiple anti-chromatin autoantibodies and splenomegaly in these mice (11-15).

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 Ifi202 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)F_1 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 *Ifi202* 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 *Ifi202* 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 *Ifi202* IS A MEMBER OF THE 200-GENE FAMILY

The gene *Ifi202* is a member of the 200-gene family (26-29). The family includes structurally related mouse genes (*Ifi202a*, *Ifi202b*, *Ifi203*, *Ifi204*, 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 melanoma-2) (26, 29). The gene *IFI16* is a human homologue of mouse *Ifi202* 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).

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 *AIM2* 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 congenic 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 congenic 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 congenic 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 C_2C_{12} mouse skeletal muscle cells, and the levels of the 202 RNA and protein are greatly increased during the differentiation of C_2C_{12} 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

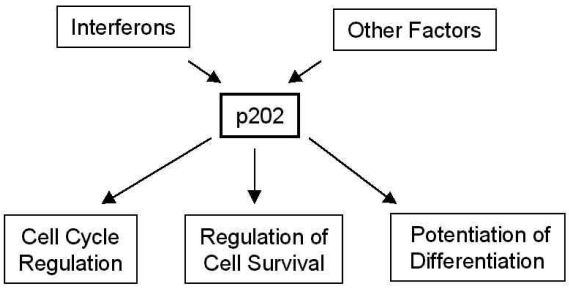

Figure 2. Hypothetical scheme for p202-mediated regulation of cell growth.

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 *Ifi202b* 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) $p21^{WAF1}$ (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 $p21^{WAF1}$ 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 $p21^{WAF1}$ could also inhibit the transcriptional activity of E2F independent of Rb (68), p202-mediated increases in the levels of $p21^{WAF1}$ could also inhibit E2F activity independent of Rb (and other pocket proteins).

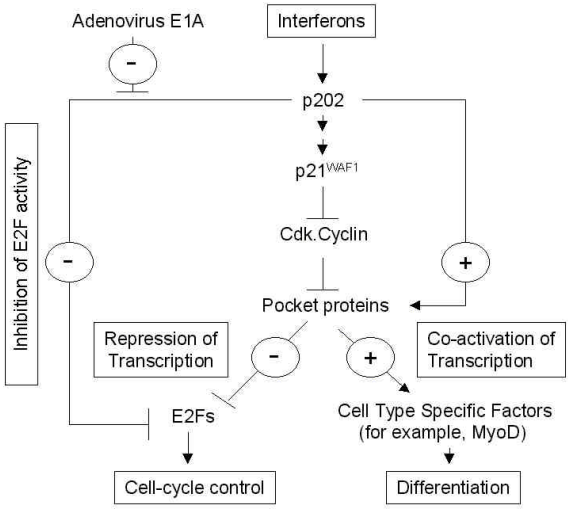

Figure 3. p202 and the Rb/E2F pathway: potential functional interactions.

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 $p21^{WAF1}$, 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

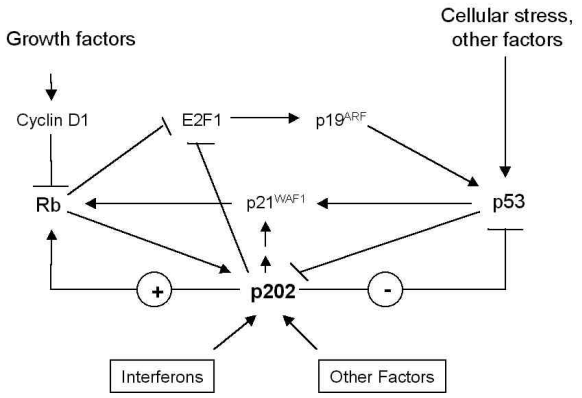

Figure 4. p202: a potential functional link between p53 and the Rb/E2F pathway.

with an expansion of particular B cell subsets, including a three-fold increase in activated B cells and greater than two-fold increase in B cells that express Fas ($CD95^+$). The accumulation of lymphoid cells in congenic mice is consistent with increased expression of p202 in B cells with inhibition of cell death contributing to increases in the number of B-lymphocytes (and T-lymphocytes) (1, 15). Because defects in apoptosis of B cells in New Zealand mouse models are also involved in the development of B cell chronic lymphocytic leukemia (B-CLL) (14), it remains to be seen whether the congenic mice develop B-CLL.

The role of p202 in inhibiting both cell proliferation and cell death may seem contradictory in the context of splenomegaly observed in the congenic mice. However, these functions of p202 may be well suited to the establishment of terminally differentiated cells that are resistant to apoptosis. Consistent with this idea, the levels of p202 increase significantly when murine myoblasts terminally differentiate *in vitro* (47) (discussed below).

It remains to be seen how increases in the levels of p202 in B cells (derived from spleens of B6.Nba2 congenic mice) inhibit apoptosis induced by anti-IgM *in vitro*. It is interesting to note that in immature B lymphoma cells, BCR engagement upon anti-IgM treatment results in growth arrest followed by apoptosis (75, 76). Importantly, the growth arrest is accompanied by activation of the pocket proteins (Rb and p130) (77, 78), inhibition of the transcriptional activity of E2F1 (78), p53-mediated increase in the levels of CDKI $p21^{WAF1}$ (79), decrease in Cdk2-associated kinase activity (75), down-regulation of cyclin D2 (76), and up-regulation of CDKI p27 (76, 80). Together, these observations provide support for the hypothesis that signaling through the BCR activates p53 and the Rb/E2F pathway. Because p202 mediates its growth-inhibitory activities, in part, through p53 and the Rb/E2F pathways and is a novel candidate for functional link between these two signaling pathways (see figure 4), it is conceivable that p202 inhibits apoptosis in B cells, in part, by modulating these two pathways.

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 C_2C_{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 C_2C_{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-

Table 1. Transcription factors modulated by p202

Transcription factor	Potential mechanism(s)	Gene(s) tested	Reference
E2F1	inhibition of DNA-binding	reporter gene, PCNA, and b-myb genes	59
E2F4	inhibition of DNA-binding	reporter gene	61
AP-1 (c-Fos and c-Jun)	inhibition of DNA-binding	reporter gene and collagenase gene	60
p53	not known	reporter genes	83
NF-kappaB (p50 and p65)	inhibition of DNA-binding	reporter gene and interferon-beta gene	60
c-Myc/Max	inhibition of dimerization	reporter gene, ornithin decarboxylase and alpha-prothymosin genes	72
MyoD	inhibition of expression and DNA-binding	reporter gene	47
Myogenin	inhibition of DNA-binding	reporter gene	47

positive thymocytes by Notch-1 signaling is consistent with its potential role in differentiation of double-positive thymocytes into either $CD4^+$ or $CD8^+$ single-positive thymocytes (49). Thus, it will be interesting to determine whether increased levels of p202 in double-positive thymocytes, for example in lupus prone strains of mice, affect the development of these T cell populations.

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).

The E2F family of transcription factors is an important regulator of cell proliferation, differentiation, and apoptosis (86). Therefore, the ability of p202 to inhibit the specific DNA-binding activity of a subset of E2F complexes makes it conceivable that p202 affects both the transcriptional activation as well as the transcriptional repression functions of E2Fs. Because E2Fs differentially regulate T lymphocyte proliferation (57), it remains to be seen whether differential regulation of the transcriptional activity of E2F factors (E2F1, -2, -3, -4, and -5) by p202 in lymphocytes contributes to a lupus phenotype (discussed below).

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 different DNA binding transcriptional regulators (67, 86). The formation of heterodimeric protein complexes has been shown to be essential for the production of high affinity E2F protein-DNA complexes since E2F homodimers have minimal DNA-binding activity and the differentiation protein homodimers have little or no affinity for DNA (86).

The E2F factors 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, Cdks, 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 immunecomplex 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

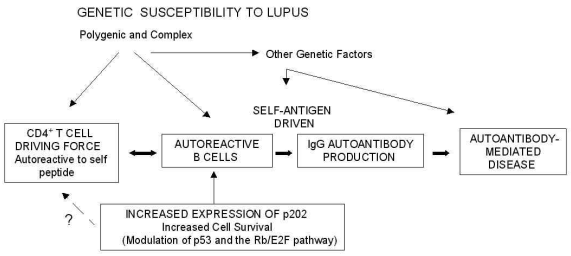

Figure 5. Hypothetical scheme for immunopathogenic process in the development of SLE and role of p202 in survival of auto-reactive B cells.

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 p53 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 *Ifi202* gene and whether additional mouse strain and/or cell type-specific transcription factors modulate the expression of the *Ifi202* 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 *Ifi202* gene expression is important to identify the physiological role of p202.

The identification of *Ifi202* 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 *Ifi202* gene contribute to differential regulation of the *Ifi202* gene and whether mouse strain, sex, and cell type-specific transcription factors contribute to regulation of *Ifi202* 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.

9. ACKNOWLEDGMENT

This work was supported by grants CA 69031 and AR 37070 from the National Institutes of Health, and a research award from the Alliance for Lupus Research (to B. L. K.). We want to thank Peter Lengyel and June Robinson for discussions and suggestions.

10. REFERENCES

1. B. L. Kotzin: Systemic lupus erythematosus. *Cell* 85, 303-306 (1996)

2. G. M. Kammer & G. C. Tsokos: Lupus: Molecular and Cellular Pathogenesis. Humana Press, Totowa, NJ (1999)

3. R. G. Lahita: Systemic Lupus Erythematosus. Academic Press, San Diego, CA ed. 3 (1999)

4. Tsokos G. C. & G. M. Kammer: Molecular aberrations in human systemic lupus erythematosus. *Mol Med Today* 6, 418- 424 (2000)

5. Marrack P., J. Kappler & B. L. Kotzin: Autoimmune disease: why and where it occurs. *Nat Immunol* 7, 899-905 (2001)

6. Vyse T. J. & J. A. Todd: Genetic analysis of autoimmune disease. *Cell* 85, 311-318 (1996)

7. Wakeland E. K., K. Liu, R. R. Graham & T. W. Behrens: Delineating the genetic basis of systemic lupus erythematosus. *Immunity* 15, 397-408 (2001)

8. Theofilopoulos A. N. & F. J. Dixon: Murine models of systemic lupus erythematosus. *Adv Immunol* 37, 269-390 (1985)

9. Kono D. H., R. W. Burlingame, D. G. Owens, A. Kuramochi, R. S. Balderas, D. Balomenos, & A. N. Theofilopoulos: Lupus susceptibility loci in New Zealand mice. *Proc Natl Acad Sci USA* 91, 10168-10172 (1994)

10. Drake C. G., S. J. Rozzo, T. J. Vyse, E. Palmer & B. L. Kotzin: Genetic contributions to lupus-like disease in (NZB x NZW)F1 mice. *Immunol Rev* 144, 51-74 (1995)

11. Vyse T. J. & B. L. Kotzin: Genetic susceptibility to systemic lupus erythematosus. *Annu Rev Immunol* 16, 261-292 (1998)

12. Kono D. H. & A. N. Theofilopoulos: Genetics of systemic autoimmunity in mouse models of lupus. *Intern Rev Immunol* 19, 367-387 (2000)

13. B. P. Tsao: Lupus susceptibility genes on human chromosome 1. Intern Rev Immunol 19, 319-334 (2000)

14. Hirose S., Y. Jiang, Y. Hamano & T. Shirai: Genetic aspects of inherent B-cell abnormalities associated with SLE and B-cell malignancy: Lessons from New Zealand mouse models. *Intern Rev Immunol* 19, 389-421 (2000)

15. Rozzo S. J., J. D. Allard, D. Choubey, T. J. Vyse, S. Izui, G. Peltz & B. L. Kotzin: Evidence for an interferon-inducible gene, Ifi202, in the susceptibility to systemic lupus. *Immunity* 15, 435-443 (2001)

16. J. U. Gutterman: Cytokine therapeutics: lessons from interferon alpha. *Proc Natl Acad Sci U S A* 91, 1198-205 (1994)

17. Stark G. R., B. R. G. Williams, R. H. Silverman, & R. D. Schreiber: How cells respond to interferons? *Ann Rev Biochem* 67, 227-262 (1999)

18. Ferrantini M. & F. Belardelli: Gene therapy of cancer with interferons: lessons from tumor models and perspective for clinical applications. *Semin Cancer Biol* 10, 145-157 (2000)

19. R. A. Rudick: Contemporary immunomodulatory therapy for multiple sclerosis. *J Neuroophthalmol* 21, 284-291 (2001)

20. Ronnblom L. & G. V. Alm: An etiopathogenic role for the type I IFN system in SLE. *Trends Immunol* 22, 427-431 (2001)

21. Blanco P., A. K. Palucka, M. Gill, V. Pascual & J. Banchereau: Induction of dendritic cell differentiation by IFN- in systemic lupus erythematosus. *Science* 294, 1540-1543 (2001)

22. Haas C., B. Ryffel & M. L. Hir: IFN-receptor deletion prevents autoantibody production and glomerulonephritis in lupus-prone (NZB x NZW)F1 mice. *J Immunol* 160, 3713-3718 (1998)

23. Lawson B. R., G. J. Prudhomme, Y. Chang, H. A. Gardner, J. Kuan, D. H. Kono & A. N. Theofilopoulos: Treatment of murine lupus with cDNA encoding IFN-gammaR/Fc. *J Clin Invest* 106, 207-15 (2000)

24. G. C. Sen: Novel functions of interferon-induced proteins. *Semin Cancer Biol* 10, 93-101 (2000)

25. D. Choubey: p202: an interferon-inducible negative regulator of cell growth. *J Biol Regul Homeost Agents* 14, 187-192 (2000)

26. Lengyel P., D. Choubey, S.-J. Li, & B. Datta: The interferon-activatable gene 200 cluster: From structure toward function. *Semin Virol* 6, 203-213 (1995)

27. Dawson M. J. & J. A. Trapani: HIN-200: A novel family of IFN-inducible nuclear proteins expressed in leukocytes. *J Leukocyte Biol* 60, 310-316 (1996)

28. Landolfo S., M. Gariglio, G. Gribaudo, & D. Lembo: The Ifi 200 genes: an emerging family of IFN-inducible genes. *Biochimie* 80, 721-728 (1998)

29. Johnstone, R. W. & J. A. Trapani: Transcription and growth regulatory functions of the HIN-200 family of proteins. *Mol Cell Biol* 19, 5833-5838 (1999)

30. Samanta H., D. D. Pravtcheva, F. H. Ruddle, & P. Lengyel: Chromosomal location of mouse gene 202, which is induced by interferons and specifies a 56.5- kDa protein. *J Interferon Res* 4, 295-300 (1984)

31. Opdenakker G., J. Snoddy, D. Choubey, E. Toniato, D. D. Pravtcheva, M. F. Seldin, F. H. Ruddle, & P. Lengyel: Interferons as gene activators: a cluster of six interferon-activatable genes is linked to the erythroid alpha-spectrin locus on murine chromosome 1. *Virology* 171, 568-78 (1989)

32. Kingsmore S. F., J. Snoddy, D. Choubey, P. Lengyel, & M. F. Seldin: Physical mapping of a family of interferon-activated genes, serum amyloid P-component, and alpha-spectrin on mouse chromosome 1. *Immunogenetics* 30, 169-74. (1989)

33. Wang H., G. Chatterjee, J. J. Meyer, C. J. Liu, N. A. Manjunath, P. Bray-Ward, & P. Lengyel: Characteristics of three homologous 202 genes (Ifi202a, Ifi202b, and Ifi202c) from the murine interferon-activatable gene 200 cluster. *Genomics* 60, 281-94 (1999)

34. Choubey D., J. Snoddy, V. Chaturvedi, E. Toniato, G. Opdenakker, A. Thakur, H. Samanta, D. A. Engel, & P. Lengyel: Interferons as gene activators. Indications for repeated gene duplication during the evolution of a cluster of interferon-activatable genes on murine chromosome 1. *J Biol Chem* 264, 17182-17189 (1989)

35. Kingsmore S. F., M. L. Watson, T. A. Howard & M. F. Seldin: A 6000 kb segment of chromosome 1 is conserved in human and mouse. *EMBO J* 8, 4073-4080 (1989)

36. Kashiwagi H. & K. Uchida: Genome-wide profiling of gene amplification and deletion in cancer. *Hum Cell* 13, 135-141 (2000)

37. Staub E., E. Dahl & A. Rosenthal: The DAPIN family: a novel domain links apoptotic and interferon response proteins. *Trends Biochem Sci* 26, 83-85 (2001)

38. Fairbrother W. J., N. C. Gordon, E. W. Humke, K. M. O'Rourke, M. A. Starovasnik, J. P. Yin & V. M. Dixit: The PYRIN domain: a member of the death domain-fold superfamily. *Protein Sci* 10, 1911-1918 (2001)

39. Aravind L., V. M. Dixit & E. V. Koonin: (2001) Apoptotic molecular machinery: vastly increased complexity in vertebrates revealed by genome comparisons. *Science* 291, 1279-84 (2001)

40. Mori J. Y., A. Rashid, B. A. Leggett, J. Young, L. Simms, P. M. Kuehl, P. Lagenberg, S. J. Meltzer, & O. Stine: Instabilotyping: Comprehensive identification of frameshift mutations caused by coding region microsatellite instability. *Cancer Res* 61, 6046-6049 (2001)

41. Gariglio M., S. Panico, G. Cavallo, D. Choubey, P. Lengyel & S. Landolfo: Impaired transcription of the poly rI:rC- and interferon-activatable 202 gene in mice and cell lines from the C57BL/6 strain. *Virology* 187,115-23 (1992)

42. Gariglio M., M. Gaboli, C. Mana, P. Foresta, G. G. Ying, A. Angeretti, D. Lembo & S. Landolfo: Characterization of nuclear factors involved in 202 gene induction by IFN-a, viruses or dsRNA in murine leukemia cells. *New Microbiol* 17, 259-267 (1994)

43. Gariglio M., M. Gaboli, C. Mana, G. G. Ying, G. Gribaudo, R. Cavallo & S. Landolfo: Characterization of nuclear factors involved in 202 gene induction by interferon-a in murine leukemia cells. *Eur J Biochem* 221, 731-739 (1994)

44. Samanta H., D. E. Engel, H. M. Chao, A. Thakur, M. A. Garcia-Blanco, & P. Lengyel: Interferons as gene activators: cloning of the 5' terminus and the control segment of an interferon activated gene. *J Biol Chem* 261, 11849-11858 (1986)

45. Gribaudo G., E. Toniato, D. A. Engel, & P. Lengyel: Interferon as gene activators. Characteristics of an interferon activatable enhancer. *J Biol Chem* 262, 11878-11883 (1987)

46. Lembo D., M. Gaboli, A. Caliendo, F. Falciani, E. Garattini & S. Landolfo: Regulation of the 202 gene expression by interferons in L929 cells. *Biochem Biophys Res Commun* 187, 628-634 (1992)

47. Datta B., W. Min, S. Burma & P. Lengyel: Increase in p202 expression during skeletal muscle differentiation: inhibition of MyoD protein expression and activity by p202. *Mol Cell Biol* 18, 1074-1083 (1998)

48. Geng Y., S. D'Souza, H. Xin, S. Walter & D. Choubey: p202 levels are negatively regulated by serum growth factors. *Cell Growth Differ* 11, 475-83 (2000)

49. Deftos M. L., E. Huang, E. W. Ojala, K. A. Forbush & M. J. Bevan: Notch1 signaling promotes the maturation of CD4 and CD8 SP thymocytes. *Immunity* 13, 73-84 (2000)

50. D'Souza S., H. Xin, S. Walter & D. Choubey D: The gene encoding p202, an interferon-inducible negative regulator of the p53 tumor suppressor, is a target of p53-mediated transcriptional repression. *J Biol Chem* 276, 298-305 (2001)

51. Choubey D. & P. Lengyel: Interferon action: cytoplasmic and nuclear localization of the interferon-inducible 52-kD protein that is encoded by the Ifi 200 gene from the gene 200 cluster. *J Interferon Res* 13, 43-52 (1993)

52. Geng Y. & D. Choubey: Differential induction of the 200-family proteins in Daudi Burkitt's lymphoma cells by interferon-alpha. *J Biol Regul Homeost Agents* 14, 263-268 (2000)

53. Liu C., H. Wang, Z. Zhao, S. Yu, Y. B. Lu, J. Meyer, G. Chatterjee, S. Deschamps, B. A. Roe & P. Lengyel: MyoD-dependent induction during myoblast differentiation of p204, a protein also inducible by interferon. *Mol Cell Biol* 20, 7024-7036 (2000)

54. Tsokos, G. C., H. K. Wong, E. J. Enyedy & M. P. Nambiar: Immune cell signaling in lupus. *Curr Opin Rheumatol* 12, 355-363 (2000)

55. Kono D. H. & A. N. Theofilopoulos: Genetic studies in systemic autoimmunity and aging. *Immunol Res* 21, 111-122 (2000)

56. Balomenos D., J. Martin-Caballero, M. I. Garcia, I. Prieto, J. M. Flores, M. Serrano, A. C. Martinez: The cell cycle inhibitor p21 controls T-cell proliferation and sex-linked lupus development. *Nat Med* 6, 171-176 (2000)

57. Murga M., O. Fernandez-Capetillo, S. J. Field, B. Moreno, L. R. Borlado, Y. Fujiwara, D. Balomenos, A. Vicario, A. C. Carrera, S. H. Orkin, M. E. Greenberg & A. M. Zubiaga: Mutation of E2F2 in mice causes enhanced T lymphocyte proliferation, leading to the development of autoimmunity. *Immunity* 15, 959-970 (2001)

58. E. L. Greidinger: Apoptosis in lupus pathogenesis. *Front Biosci* 6, d1392-1402 (2001)

59. Choubey D., S. J. Li, B. Datta, J. U. Gutterman & P. Lengyel: Inhibition of E2F-mediated transcription by p202. *EMBO J* 15, 5668-5678 (1996)

60. Min W., S. Ghosh, & P. Lengyel: The interferon-inducible p202 protein as a modulator of transcription: inhibition of NF-kappa B, c-Fos, and c-Jun activities. *Mol Cell Biol* 16, 359-368. (1996)

61. Choubey D. & J. U. Gutterman: Inhibition of E2F-4/DP-1-stimulated transcription by p202. *Oncogene* 15, 291-301 (1997)

62. Yan D. H., Y. Wen, B. Spohn, D. Choubey, J. U. Gutterman & M. C. Hung: Reduced growth rate and transformation phenotype of the prostate cancer cells by an interferon-inducible protein, p202. *Oncogene* 18, 807-811 (1999)

63. Wen Y., D. H. Yan, B. Spohn, J. Deng, S. Y. Lin & M. C. Hung: Tumor suppression and sensitization to tumor necrosis factor alpha-induced apoptosis by an interferon-inducible protein, p202, in breast cancer cells. *Cancer Res* 60, 42-46 (2000)

64. Wen Y., D. H. Yan, B. Wang, B. Spohn, Y. Ding, R. Shao, Y. Zou, K. Xie, M. C. Hung: p202, an interferon-inducible protein, mediates multiple antitumor activities in human pancreatic cancer xenograft models. *Cancer Res* 61, 7142-7147 (2001)

65. Koul D., R. Lapushin, H. J. Xu, G. B. Mills, J. U. Gutterman & D. Choubey: p202 prevents apoptosis in murine AKR-2B fibroblasts. *Biochem Biophys Res Commun* 247, 379-82 (1998)

66. Gutterman J. U. & D. Choubey: Retardation of cell proliferation after expression of p202 accompanies an increase in $p21^{WAF1/CIP1}$. *Cell Growth Differ* 10, 93-100 (1999)

67. J. R. Nevins: Toward an understanding of the functional complexity of the E2F and retinoblastoma families. *Cell Growth Differ* 9, 585-93 (1998)

68. Dimri G. P., M. Nakanishi, P. Y. Desprez, J. R. Smith, J. Campisi: Inhibition of E2F activity by the cyclin-dependent protein kinase inhibitor p21 in cells expressing or lacking a functional retinoblastoma protein. *Mol Cell Biol* 16, 2987-97 (1996)

69. Choubey D. & P. Lengyel: Binding of an interferon-inducible protein (p202) to the retinoblastoma protein. *J Biol Chem* 270, 6134-40 (1995)

70. Hertel L., S. Rolle, M. De Andrea, B. Azzimonti, R. Osello, G. Gribaudo, M. Gariglio & S. Landolfo: The retinoblastoma protein is an essential mediator that links the interferon-inducible 204 gene to cell-cycle regulation. *Oncogene* 19, 3598-608 (2000)

71. Xin H., S. D'Souza, L. Fang, P. Lengyel & D. Choubey: p202, an interferon-inducible negative regulator of cell growth, is a target of the adenovirus E1A protein. *Oncogene* 20, 6828-6839 (2001)

72. Wang H., C. Liu, Y. Lu, G. Chatterjee, X. Y. Ma, R. N. Eisenman & P. Lengyel: The interferon- and differentiation-inducible p202a protein inhibits the transcriptional activity of c-Myc by blocking its association with Max. *J Biol Chem* 275, 27377-27385 (2000)

73. P. E. Lipsky: Systemic lupus erythematosus: an autoimmune disease of B cell hyperactivity. *Nat Immunol* 2, 764-766 (2001)

74. Su L. & M. David: Inhibition of B cell receptor-mediated apoptosis by IFN. *J Immunol* 162, 6317-6321 (1999)

75. Joseph L. F., S. Ezhevsky & D. W. Scott: Lymphoma models for B-cell activation and tolerance: anti-immunoglobulin M treatment induces growth arrest by preventing the formation of an active kinase complex, which phosphorylates retinoblastoma gene product in G1. *Cell Growth Differ* 6, 51-57 (1995)

76. Benerji L., J. Glassford, N. C. Lea, N. S. B. Thomas, G. G. B. Klaus & E. W.-F. Lam: BCR signals target p27 and cyclin D2 via the PI3-K signaling pathway to mediate cell cycle arrest and apoptosis of WEHI 231 B cells. *Oncogene* 20, 7352-7367 (2001)

77. Maheswaran S., J. E. McCormack & G. E. Sonenshein: Changes in phosphorylation of myc and Rb antioncogene protein products during growth arrest of the murine lymphoma WEHI 231 cell line. *Oncogene* 6, 1965-1971 (1991)

78. Lam E. W., M. S. Choi, J. van der Sman, S. A. Burbidge & G. G. Klaus: Modulation of E2F activity via signaling through surface IgM and CD40 receptors in WEHI-231 lymphoma cells. *J Biol Chem* 273, 10051-10057 (1998)

79. Wu M., R. E. Bellas, J. Shen & G. E. Sonenshein: Roles of the tumor suppressor p53 and the cyclin-dependent kinase inhibitor p21 in receptor-mediated apoptosis of WEHI 231 B lymphoma cells. *J Exp Med* 187, 1671-1679 (1998)

80. Wu M., R. E. Bellas, J. Shen, W. Yang & G. E. Sonenshein: Increased p27 cyclin-dependent kinase inhibitor gene expression following anti-IgM treatment promotes apoptosis of WEHI 231 B cells. *J Immunol* 163, 6530-6535 (1999)

81. A. J. Levine: p53, the cellular gatekeeper for growth and division. *Cell* 88, 323-331 (1997)

82. Ryan K. M., A. C. Phillips & K. H. Vousden : Regulation and function of the p53 tumor suppressor protein. *Curr Opin Cell Biol* 13, 332-337 (2001)

83. Datta B., B. Li, D. Choubey, G. Nallur, & P. Lengyel: p202, an interferon-inducible modulator of transcription, inhibits transcriptional activation by the p53 tumor suppressor protein, and a segment from the p53-binding protein 1 that binds to p202 overcomes this inhibition. *J Biol Chem* 271, 27544-27555 (1996)

84. Donehower L. A., M. Harvey, B. L. Slagle, M. J. McArthur, C. A. Montgomery Jr, J. S. Butel & A. Bradley: Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumors. *Nature* 356, 215-221 (1992)

85. Yee A. S., H. H. Shih & S. G. Terosien: New perspective on retinoblastoma family function in differentiation. *Front Biosci* 3, d532-547 (1998)

86. Muller H. & K. Helin: The E2F transcription factors: key regulators of cell proliferation. *Biochim Biophys Acta* 1470, M1-12 (2000)

Key Words: Lupus-susceptibility, Autoantibodies, Interferons, The 200-family, Interferon-inducible p202, Cell cycle, Retinoblastoma, E2F, p53, $p21^{WAF1}$, Apoptosis, Differentiation, Review

Send correspondence to: Divaker Choubey, Ph. D., Department of Radiation Oncology, Loyola University Medical Center & Hines V. A. Hospital, Mail code: 114B, Hines, IL 60141, USA, Tel.: 708-202-8387 x23407, Fax: 708-202-2647, E-mail: dchoube@lumc.edu