*Review*

# A new approach of abnormal apoptosis as a cause of autoimmunity and malignancy

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**Auto-reactive cells which escape from natural apoptosis represent a continuous threat of potential autoimmune response. Abnormal apoptosis can play a role in negative selection of B and T lymphocytes that escaped the self-reactive nature, and so, apoptosis could represent an additional source of auto-antibody. Increased activity of T cells (CD3+, CD4+, or Th1 helper)) will, at a high serum level, cause a high expression of various types of inflammatory interleukins: IL1-β, IL2. The most important regulatory mechanisms of apoptosis in T and B cells are: death receptor cells, CD 95(Fas), TNF-tumor necrosis, caspases, family Bcl-2 proto-oncogenes, Bax gene, p53 tumor suppressor gene, and NF-κB nuclear factor of transcription. The "decision" to undergo programmed cell death is made only in the presence of extrinsic or intrinsic apoptotic messengers. Extrinsic inductors are ligands – cytokines – that bind to death receptors (DRs) found on the cells' surface, while intrinsic inductors come from the mitochondria or from the nucleus cells.**

**Key words:** Tumor suppressor gene P53, rheumatoid arthritis, systemic lupus erythematosus, tumor necrosis factor, zeta-chain-associated protein kinase 70.

## INTRODUCTION

Apoptosis and autoimmunity share essential similarities in their cell-damage mechanisms. However, apoptosis is a normal process which ensures the permanent renewal of cells, while autoimmunity is an abnormal process that can lead to serious health issues. (Grodzitsky and Elcon, 2002).

## EXTRINSIC PATHWAY OF APOPTOSIS

Apoptosis is induced by the signal molecules – known as ligands – which are released by other cells, and which bind to the trans-membrane death receptors of the target cell. For example, the immune system's natural killer cells posses the Fas ligand (FasL) on their surface: the binding of the FasL to Fas receptors (a death receptor) on various target cells will trigger the aggregation of multiple receptors on the surface of that target cell (Saratoy et al., 2002). The aggregation of these receptors then leads to the recruitment of an adapter protein, known as Fas-associated death domain protein (FADD), on the cytoplasmic side of the receptors. FADD, in turn, recruits caspase-8 (an initiator protein), forming the death-inducing signal complex (DISC).

## INTRINSIC PATHWAY OF APOPTOSIS

The intrinsic pathway is triggered by cellular stress – specifically, mitochondrial stress caused by various factors, such as DNA damage. The stress signal will cause the pro-apoptotic proteins found in the cytoplasm – BAX (pro-apoptotic, cytoplasmic protein) and BID – to bind to the outer membrane of the mitochondria and signal the release of the internal mitochondrial content.

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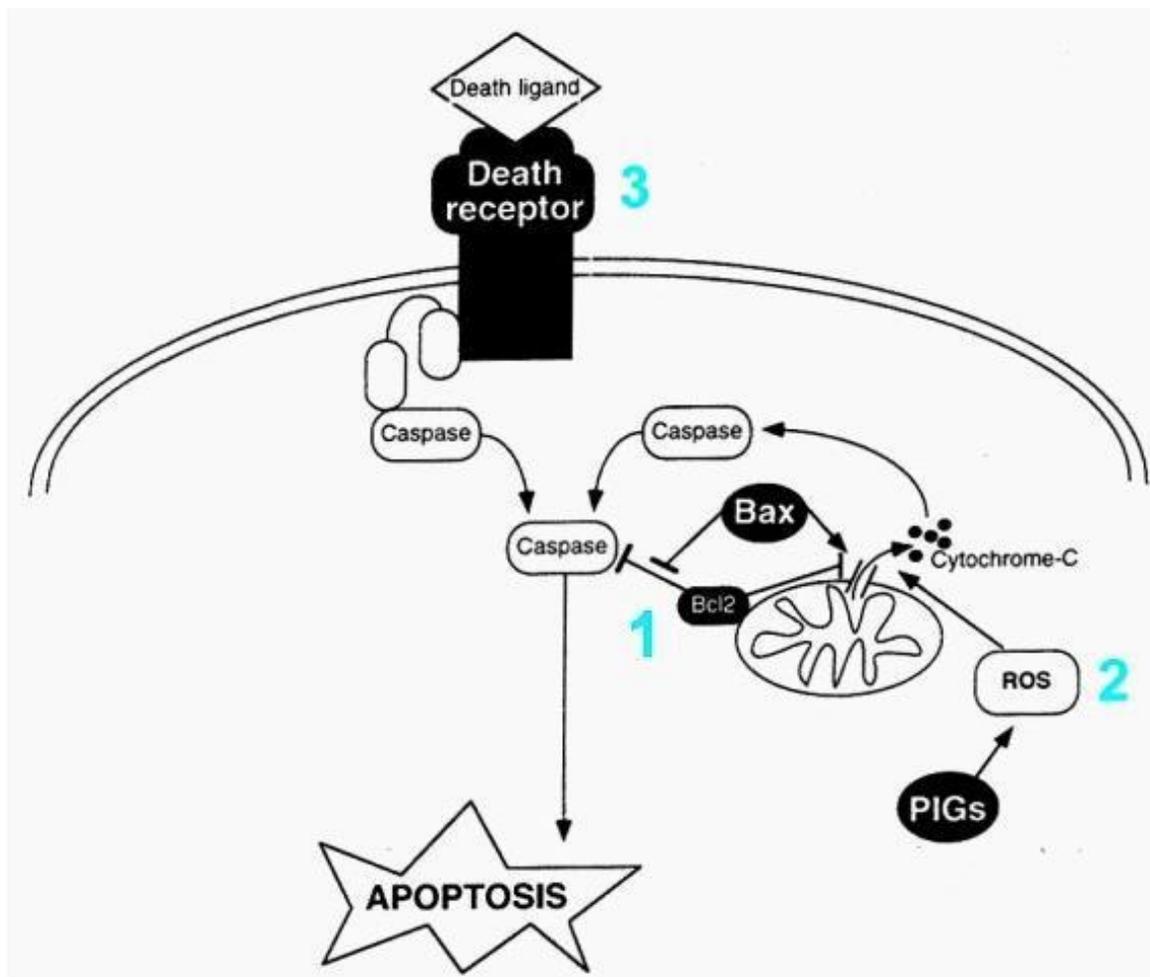
**Figure 1.** The p53 protein binds to Bcl-2 family proteins and increases the mitochondria permeability; this causes the release of the mitochondrial contents which will trigger the apoptotic cascade. Tumor-associated mutations of the p53 gene will inactivate this pathway and inhibit apoptosis. Therefore, these interactions are important in inhibiting tumorigenesis.

However, the signal of BAX and BID is not enough to trigger a full release of the mitochondrial content: BAK, a pro-apoptotic protein found in the mitochondria, is also needed to fully promote the mitochondrial release; it is important to note that the mitochondrial content also includes cytochrome C. (Figure 1). Besides cytochrome C, the mitochondrial content released also contains the apoptosis inducing factor (AIF) which facilitates DNA fragmentation, preventing the activity of the inhibitors of apoptosis (IAP) (Warrell et al., 2003; Yau, 2001; Mary and Collins, 1993).

**CASPASE ACTIVITY**

Caspase activity in apoptotic cells may lead to a presentation of cryptic epitopes or neo-epitopes to which the immune system is not tolerant. Citrulinated, acetylated, and phosphorylated forms of antigens may lead to increased immunogenicity. The initiator procaspase-9 is the main upstream enzyme in the apoptotic cascade. Down the enzymatic cascade, there is an amplification of the initial caspase activity until the key reactions are reached: cleavage of proteins that normally hold DNAase in its inactive form, cleavage of poly-ADP-ribose-polymerase, cleavage of nuclear laminas. Mitochondria have a fundamental position in executing apoptosis induced by intracellular signals. When cells are stressed due to physical signals, chemical stimulus, hypoxia, or cytokines, mitochondria release pro-apoptotic proteins, including cytochrome C. Following its release into the cytoplasm, cytochrome C forms a complex with the high-energy-molecule, adenosine triphosphate (ATP) and with the enzyme Apaf-1. In turn, this newly formed complex will activate caspase-9, an initiator protein, which then interacts with the cytochrome C-ATP-Apaf-1 complex to form an apoptosome. The apoptosome activates caspase-3, the effector protein which then
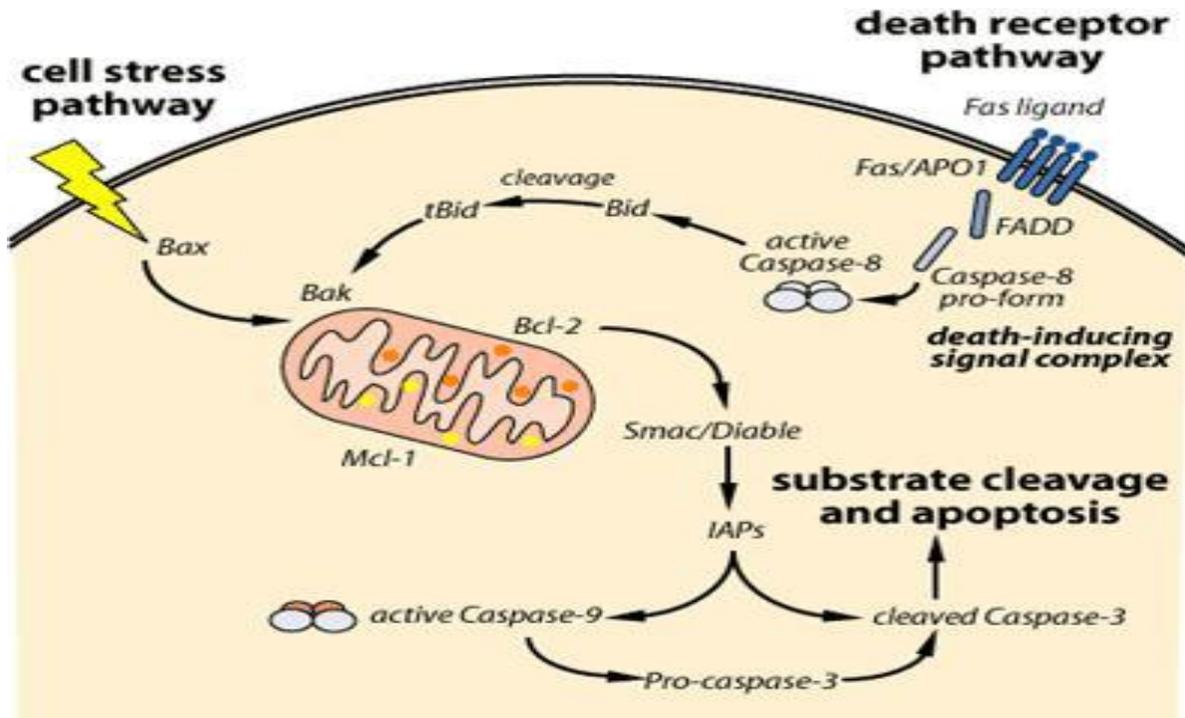
**Figure 2.** The intrinsic and extrinsic pathways leading to apoptosis.

initiates the cellular degradation (Figure 2).

## APOPTOSIS AS A CAUSE FOR CANCER

A dysfunctional apoptotic pathway may lead to the development of cancers. Due to the sensitivity of the intrinsic pathway, tumors arise more often through the intrinsic pathway than the extrinsic pathway (Martin, 1998).

A very common cause of malignant tumors through the intrinsic pathway is a mutation in the p53 protein (tumor-suppressor protein). Besides regulating apoptosis, p53 also regulates the checkpoints of the cell cycle, DNA repair, senescence, and genomic integrity (Csipo et al., 1998). A mutation causes the p53 gene to lose any of its functions will inevitably lead to carcinogenesis by letting the cell grow indefinitely, without any regulation. Another important factor in carcinogenetic process is the balance between the pro-apoptotic and anti-apoptotic members of the Bcl-2 family. In a tumor cell, a mutation in the Bcl-2 gene results in increased expression will suppress the normal function of the pro-apoptotic proteins BAX and BAK, leading to malignancy (Martin, 1998).

On the other hand, a mutation in the BAX or BAK genes can cause a down-regulation of expression, causing the cell to lose the ability to regulate apoptosis, once again, leading to cancer cells (Martin, 1998). The inhibitor of apoptosis (IAP) family genes, which encode

negative regulatory proteins, can prevent apoptotic cell death (Figure 3). In the normal cell, the p53 protein binds DNA, stimulating another gene to produce a protein called p21, which interacts with a cell division stimulating protein (cdk2) (Udristioiu et al., 2010). When p21 forms a complex with cdk2, the cell cannot pass through to the next stage of cell division, and remains arrested in G1 (Schmitt et al., 2002). The p53 protein product of a *TP53* mutant gene cannot bind DNA in an effective way, and as a consequence, the p21 protein is not made available to act as the stop signal for the cell cycle/cell division. Therefore, cells divide uncontrollably and form tumors. (Yau, 2001).

Not surprisingly, there is an increased frequency in the amplification of the *MDM2* (p53 inhibitor) gene in many human cancers. MDM2 (ubiquitin ligases protein) is involved in the mechanism for the down-regulation of p53 activity through ubiquitin-dependent proteosomal degradation of p53 (Martin, 1998).

## NEW CANCER THERAPIES

In experimental models, disrupting the MDM2–p53 interaction restored p53 function and sensitized tumors to chemotherapy or radiotherapy. (Kojima et al., 2005). This strategy could be particularly beneficial in treating cancers that do not harbor *TP53* mutations. For example in hematologic malignancies, such as multiple myeloma,
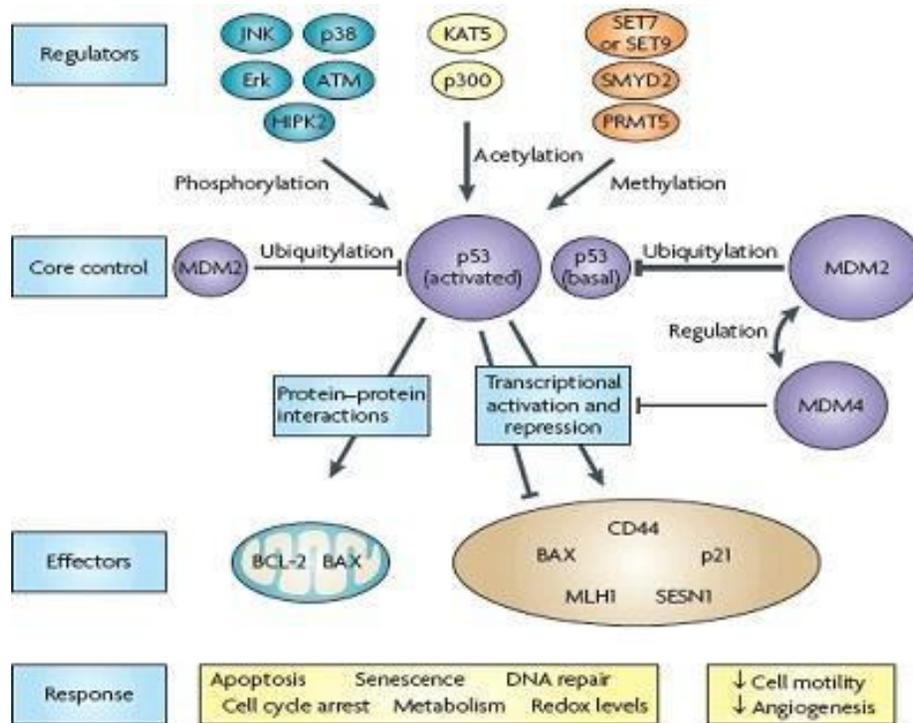
**Figure 3.** .Activated p53 protein regulates the apoptosis by activating of same pro-apoptopic proteins as BAK and by prevention of activated proto-oncogene BCL-2 and MDM2 protein. Activity of p53 is dependent on its levels. These levels are tightly controlled through covalent modifications. Stress sensors of the cell determine the phosphorylation, ubiquitylation, methylation, or acetylation of p53. These modifications elicit cascade events that counteract the deleterious effects of DNA damage, hypoxia, metabolic stress, or oncogene activation.

chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), and Hodgkin's disease, the induction of p53 – using a small MDM2-inhibitor molecule, nutlin-3 – can induce the apoptosis of malignant cells. Nutlins are a group of cis-imidazoline analogs, first identified by Vassilev et al. (2004), which have a high binding potency and selectivity for MDM2. Crystallization data have shown that nutlin-3 mimics the three residues of the helical region of the trans-activation domain of p53 (Phe19, Trp23 and Leu26), which are conserved across species and critical for binding to MDM2 (Wade et al., 2010). Nutlin-3 displaces p53 by competing for MDM2 binding. It has also been found that nutlin-3 potently induces apoptosis in cell lines derived from hematologic malignancies, including AML, myeloma, ALL, and B-cell CLL (Secchiero et al., 2010).

A large cohort study of primary CLL, done on over 100 patients, examined the samples from the patients for a response to MDM2 inhibition. The study found direct correlation between wild-type *TP53* status and MDM2 inhibitor-induced (nutlin-3 and MI-219) cytotoxicity across various CLL subtypes. This response was not predicted by other biomarkers used clinically for CLL, including in B cells, expression of the zeta-chain-associated protein

kinase 70 (ZAP70), un-mutated immunoglobulin variable genes, and mono-allelic *ATM* (ataxia telangectazica mutated gene) loss. The protein ZAP70 is a member of the protein-tyrosine kinase family. ZAP70 is normally expressed in T cells and natural killer cells, and has a critical role in the initiation of T-cell signaling. ZAP70 in B cells is used as a prognostic marker in identifying different forms of chronic lymphocytic leukemia (CLL). Some studies showed that patients with cancer make antibodies against p53 proteins, but the frequency and magnitude of this response is still under debate (Vojtesek et al., 1995). However, a large number of patients with cancer did produce p53-reactive T cells (Van der Burg et al., 2001).

The results from these studies served as a good reason to attempt the vaccination of patients using p53-derived peptides, and a several clinical trials are currently in progress. The most advanced work used a long synthetic peptide mixture derived from p53 (p53-SLP; ISA Pharmaceuticals, Bilthoven, the Netherlands) (Speetjens et al., 2009; Shangary et al., 2008; Van der Burg et al., 2001). The vaccine is delivered in the adjuvant setting and induces T helper type cells. However, the response may not be potent enough to result in clinical benefit as a mono-therapy: most patients had T-helper cells that failed

to produce key cytokines. This indicated that these p53-specific T-helper responses are not polarized. Therefore, approaches are being investigated to promote a stronger and more correctly polarized response using both DNA-based and dendritic cell-delivered p53 vaccines.

## FasL AND OXIDATIVE STRESS IN APOPTOTIC EVENTS

The main death receptor (DR), named CD95 (Fas), as well as CD 120a (TNF-R1), DR3, DR4, DR5, and DR6, are responsive to cytokines belonging to the tumor necrosis factors (TNF-α, lymphotoxin, Fas ligand (FasL}, Apo-13). The link between apoptosis and TNF activity shows why abnormal production of TNF plays an important role in several autoimmune diseases: rheumatoid arthritis (RA), multiple sclerosis (MS), diabetes mellitus, ulcerative colitis (Topic, 2005).

Fas ligand (FasL) is a type II membrane protein which belongs to the tumor necrosis factor (TNF) family. FasL induces apoptosis in target cells bearing the receptor Fas. The role of the Fas-FasL system has been best characterized in the immune system: interactions between Fas and FasL are functionally involved in tissue-specific regulation of various immune processes: for example, FasL expression has been detected in immune-privileged organs, such as the eye and the testis, which are protected from destructive immune responses by inducing the apoptosis of infiltrating Fas-bearing immune cells. Endothelial cells express Fas, but are normally resistant to Fas-mediated apoptosis (Saratoy et al., 2002; Scaffidi et al.,1998).

Oxidative stress has been shown to alter various aspects of endothelial functions: increase in endothelial adhesiveness to neutrophils via protein-kinase-C-activation-dependent pathways, increase in the production of platelet-activating factor, as well as in the expression of intracellular adhesion molecule-1. Thus, up-regulation of FasL expression on the endothelium may contribute to anti-inflammatory reactions by reducing leukocyte transmigration in tissues. Recent and previous studies have shown that increased oxidative stress induces FasL expression by T-lymphocytes, microglial cells, and intestinal epithelial cells, suggesting that oxidative stress is involved in the FasL-mediated apoptotic mechanism of Fas-bearing target cells (Adrain et al., 2002; Csipo et al., 1998; Suzuki et al., 2006; Scaffidi et al., 1998).

$H_2O_2$ is one of the most important oxidant agents derived from leukocytes and endothelial cells. It exerts a toxic effect on susceptible cells at high concentrations, but alters cell functions at low concentrations, by modulating signal transduction pathways in certain cells, including endothelial cells (Suzuki et al., 2006). Cigarette smoke is an important source of oxidative agents, including $H_2O_2$, and is thought to be a significant risk

factor for chronic endothelial damage leading to atherosclerosis (Ramage et al., 2006; Bauer et al., 1998). However, the mechanism for oxidative-stress-induced FasL expression is still unclear. A previous report documented an association between oxidative-stress-induced FasL expression and the NF-κB nuclear transcription factor. The functional role of NF-κB has not been fully demonstrated (Laur et al., 2010). Two NF-κB binding sites are located at positions -537 to -521 and -57 to -47, respectively, relative to the transcription start site of the human CD95L promoter (Diogo et al., 2010). High levels of soluble CD95 were found in rheumatoid arthritis (RA): these high levels contribute to the inhibition of apoptosis of synoviocytes and inflammatory cells. An inadequate apoptosis due to defective CD95 may promote an extended survival of synoviocytes; additionally their responsiveness to CD95L is decreased by TGFβ, IL1-β, and TNF-α. Simultaneously, expression of CD95 and its ligand causes apoptotic cells death by paracrine or autocrine mechanism and during inflammation, IL1-β and interferon-1α induce massive CD up-regulation (Livolsi et al., 2001). High rate of apoptosis can overload the phagocytic capacity and may trigger an autoimmune reaction, through the presentation of nucleosomes to the immune system. Apoptosis also plays a role in negative selection of B and T lymphocytes that escaped the self-reactive nature; again, in this case, apoptosis can be a source of auto-antibodies. Elevated activity of the receptor CD3+, CD4+ or Th1 helper cells will be induced by high serum level of interleukins (ILs): IL1-β, IL2 and TNF (Topic, 2005).

## APOPTOSIS AS A CAUSE FOR AUTO-IMMUNITY

In various cell types, tumor necrosis factor (TNF-α) induces either cell death, or mitogenesis, through different signaling pathways. The blockade of TNF-α, induced $K^+$ channel activity effectively and prevented NF-κB nuclear translocation and binding to DNA, diminishing the cells survival (Ling et al., 2005). Inhibition of $K^+$ channel activity with specific channel blockers results in attenuation of the cell cycle in the $G_1$ phase. TNF-α also induces mRNA expression of various $K^+$ channel types during a systemic inflammatory response, as well as tumor cell proliferation in brain and other cancer types. In the case of systemic lupus erythematosus (SLE), more than 40 genes have been identified as possible causes for the disease. There are three different groups of genes that are responsible for pathogenic process. A category of genes codes for molecules that have an impact on the clearance of apoptotic cells. When these accumulate, the production of ANA by auto-reactive lymphocytes is stimulated. The second group codes for molecules that may act be thwarting the deletion of self-reacting B cells and T cells (Kaplan, 2004). The third group codes for molecules that amplify or modulate lymphocyte signaling and expansion. The studies performed in the last 10

years suggest that the neonatal wave of beta cells apoptosis might activate auto-antigens necessary for triggering Th-4 in systemic lupus erythematous (Illei et al., 2004).

In systemic auto-immune diseases, in particular SLE, auto-antibodies have been found against a range of intra-cellular antigens. This suggests that the humoral response may be driven by products from apoptotic cells, highlighting the nucleosomes as self-generating potential antigens. The antinuclear antibodies (ANA) may be present in response to antigens such as double-stranded DNA (dsDNA), extractable nuclear antigen Sm (UI RNP), nucleosomic (chromatin) antigen mixture of DNA molecules, histones (H2A, H2B, H3, H4), and nucleoplasma nucleomatrix antigens: SS-A, and SS-B. The production of TNF plays an important role in several autoimmune diseases, such as: rheumatoid arthritis, multiple sclerosis, diabetes mellitus, ulcerative colitis.

## CONCLUSION

Apoptosis has traditionally been thought of as non-inflammatory process which does not induce an immune response. However, recent studies indicate that apoptotic cells can be involved in auto-immune processes. In some cases the cells can display auto-reactive antigens on their surface blebs, can activate T cells and B cells, and can induce the formation of auto-antibodies.

The most important cell-regulatory mechanisms of apoptosis in mammalian T cells and B cells are: death receptors, caspases, mitochondria, the Bcl-2 family proto-oncogene, tumor suppressor gene *TP53*, TNF, and nuclear translocation factor, NF-Kb. These immune responses to p53 proteins occur most frequently in patients with high p53 protein expression in their tumors, although they are also rarely seen in patients with low p53 expression, and even in healthy individuals.

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