Review Article:
THE POTENTIAL INTERVENTION ON APOPTOSIS AND CELL CYCLE ACTIVITY IN RHEUMATOID ARTHRITIS

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ABSTRACT

RA (rheumatoid arthritis) is a chronic progressive polygenic multifactor autoimmune disease with a high socio-economic burden. The regulation of cell death and proliferation is vital for homeostasis, but the mechanisms that coordinately balances these two events in rheumatoid arthritis (RA) remains largely unknown. In RA, the synovial lining increases through decreased cell death e, and/or enhanced proliferation. The aberrant decrease in apoptosis and/or increased cell cycle activity of the fibroblast-like and macrophage-like synoviocytes is responsible for the synovial hyperplasia and contributes to the destruction of cartilage and bone. Besides synovial cells, T cells, and mast cells also increase in number and life span, resulting in the increase of macrophage activation, and production of autoantibodies. Recently, numerous molecules that modulate apoptosis and cell cycle have been implicated to play a role in RA. This review will describe the current understanding of the molecular mechanisms that govern apoptosis and cell cycle, their relationship to RA pathogenesis, and the potential intervention in the treatment of RA.

Keywords*: potential intervention, apoptosis, cell cycle, Rheumatoid Arthritis*

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APOPTOSIS

Apoptosis is a physiologic programmed cell death. It plays a central role both in development and in homeostasis of metazoans. Cells die by apoptosis in the developing embryo during morphogenesis in the adult animal, during tissue turnover or at the end of an immune response. Because the physiological role of apoptosis is crucial, aberration of this process can be detrimental (Ashkenazi 1998). Thus, unscheduled apoptosis of certain cells in joints may contribute to disorders such as AR (Tak 1999). Apoptosis is a tool to control quality and quantity of cells in all tissues. Excess number of cells is catastrophic. There are 2 pathways of apoptosis mechanisms: First, intrinsic pathway. Going through stimulation of intracellular receptors such as activated apoptotic protease-activating factor 1 (Apaf-1), which leads to binding of Apaf-1 to its ligand, cytochrome C that released by mitochondrial injury asken. This is also called as mitochondrial pathway. Second, extrinsic pathway. Going through death receptors (DRs) on the cell surface. This is also called as ligand-dependent triggering of death receptors (Ashkenazi 1998; Perlman 2001a; Perlman 2001b).

INTRINSIC PATHWAY (MITOCHONDRIAL PATHWAY)

The intrinsic pathway of apoptosis is mediated by the Bcl-2 protein family. Bcl-2 (B-cell follicular lymphoma-2). Additional apoptotic accelerators (Bax, Bak, Bid, Bim, Mtd, Bik, Bok, Bip, Diva, Hrk, Bcl-xS, or Blk) and protectors (Bcl-xL, Mcl-1, A1 and Bcl-w) that share homologous regions with Bcl-2 have also been identified (Perlman 2001a). Bcl-2-related proteins, induces or inhibits apoptosis that proceeds through the intrinsic pathway. Bcl-2-related proteins, in general, are targeted to the mitochondria suggesting that mitochondrial dysfunction is involved in apoptosis (Ashkenazi 1998; Perlman 2001b; Schirmer 1998). During apoptosis, the barrier functions of mitochondrial membranes are lost, leading to the dissipation of the inner transmembrane potential In addition, there is a release of intermembrane mitochondrial proteins, including cytochrome C, Smac/Diablo and AIF. Cytochrome C released into the cytosol activates the initiator caspase 9 following the formation of the “apoptosome”, which consists of the adaptor protein Apaf 1, cytochrome C, pro-caspase 9 and dATP. Caspase 9 activates caspases 3 and 7, which mediate the downstream degradative events of

apoptosis. These events are prevented by Bcl-2/BclxL, while Bax, Bak or Bid induces the cytochrome c release (Aman 2001), although the mechanism remains obscure. However, recent studies employing Bax/Bak double knockout mouse embryonic fibroblasts reveal that mitochondrial-mediated apoptosis requires both Bax and Bak regardless of the stimuli. Apoptosis mediated by the death receptor pathway may be blocked at the death inducing signaling complex (DISC) by the naturally occurring dominant negative caspase 8 termed Flice inhibitory protein (Flip) (Fujii 2002; Smith 2004; Yang 2005). Flip binds to procaspase 8 through the death effector domains (DED) and prevents its association with FADD, thereby inhibiting the transmission of the Fas death signal at the most proximal point (Figure 1). Direct inhibition of Fas, TRAIL-RI and RII, or TNFR1-mediated apoptosis is mediated by Flip, since mouse embryonic fibroblasts deficient in Flip were sensitive not only to Fas, but also to TNF-α mediated apoptosis, demonstrating that Flip is essential for the inhibition of death receptor (Ashkenazi 1998; Ichikawa 2003; Perlman 2001b; Smith 2004).

EXTRINSIC PATHWAY (DEATH RECEPTOR PATHWAY)

The induction of apoptosis mediated by the extrinsic pathway is initiated by ligation of death receptors at the cellular surface to their cognate ligands (Ashkenazi, 1998). Members of the TNF superfamily including Fas, TRAIL RI (DR4) and RII (DR5), TNF-α receptor (TNFRI) are ubiquitously expressed type I membrane receptor proteins that share a homologous region (death domain) and have been shown to induce apoptosis when overexpressed. The TNF superfamily also consists of the cognate ligands for the death receptors, which are type II membrane proteins and include Fas ligand (FasL), TNF, and TRAIL. Oligomerization of the death receptors by their ligands induces the recruitment of both Fas-associated protein with death domain (FADD) (Ashkenazi A, 1998, Schirmer M, 1998, Smith MD, 2004) and the precursor form of the cysteine protease caspase 8 (FLICE/MACH) (Yamasaki S, 2001) to the C-terminus of Fas, forming the death inducing signaling complex (DISC) (Kawakami 2004; Panayi 2001; Smith 2004; Yang 2004). Aggregation or oligomerization of pro-caspase 8 results in its autocatalysis/activation and the initiation of the cell death pathway. Apoptosis may then directly proceed to the activation of the effector caspases 3 and 7 (Figure 1). An additional pathway of death receptor-induced cell death may proceed through the mitochondrial pathway by activating Bid, a Bcl-2-related protein, which is cleaved by caspase 8 following death receptor ligation. Cleaved Bid is targeted to the mitochondria and ultimately results in the Induction of apoptosis mediated by the mitochondrial apoptotic pathway (Figure 1). Although previous investigations demonstrate conflicting results concerning Bcl-2/Bcl-xL inhibition of death receptor-induced apoptosis, a recent study suggests that the ability of Bcl-2/Bcl-xL to suppress death receptor-mediated apoptosis depends on the levels of caspase 8 and FADD (Ichikawa 2003; Perlman 2001a; Smith 2004; Yang 2004).

POTENTIAL APPROACH TO INDUCE APOPTOSIS IN RA

Studies using in situ end labeling (ISEL), TdT-nick end labeling (TUNEL) and electron microscope on the synovial membrane of patients with RA, showed decrease of DNA fragmention, which is identical to decrease of apoptosis of synovial cells (Kitagawa 2005; Krause 2002; Perlman 2001b; Smith 2004). The elevated levels of inflammatory mediators, mainly IL-1β and TNF-α (Dinarello 2001; Juurikivi 2005) and growth factors are related with decrease of apoptosis, which cause synovial hyperplasia. In RA, IL-1β and TNF-α suppress apoptosis through the upregulation of a Bcl-2-related protein (Perlman 2001a). TNF-α may also inhibit the binding of Fas and FasL, resulting in decrease of apoptosis (Yamasaki 2001). Another problem, high levels of TNF-α is not able to elicit apoptotic signaling through TNFRs on synovial cells. This is probably related to counterbalance of concomitant elevation of NFκB levels. Several approaches which potentially induce apoptosis in RA are:

1. Inhibition of Flip expression
   A synthetic bisindolylmaleimide I was reported to inhibit Flip expression from macrophage sinovial and increases ligation Fas and FasL, in order to induce intrinsic pathway (Catrina 2001).
2. Induction of Bax activation with Cathepsin D (Bidère 2003).
3. Inhibition on *c-myc* to increase apoptotic molecules like Bcl2 (Perlman 2001a).
4. Induction of TRAIL to increase apoptosis of synovial cells, with an anti fungal, trichosatatin A (Jüngel 2005)
5. Inhibition of nuclear factor-κB (NFκB) may be able to
   a. Induce intrinsic pathway through inhibition of anti-apoptotic molecules such as Bcl-2, Bcl-xL
   b. Induce extrinsic pathway through down regulation of X-linked inhibitor of apoptosis (XIAP) expression, which is followed by

increseased caspase 3 activities (Yamasaki 2001; Smith 2004)

6. Inhibits tumor supresor gene like PTEN (phosphatase and tension homologue) leads to inhibition of Akt-1 and decrease of survival of synovial fibroblasts and synovial macrophage (Weng 2001).
7. Inhibition of c-kit tyrosine kinase with immatinib which leads to apoptosis of mast cells (Juurikivi 2005)
8. Activation of PPAR-γ which is abundantly expressed by macrophages and fibroblasts may induce apoptosis through unclear mechanisms in RA. Activator of PPAR-γ may be able to increase apoptosis in RA (Yamasaki 2001).
9. CD44 is a ubiquitous molecule known as a hyaluronan receptor. However, the relevance of CD44 to inflammatory processes, for example, rheumatoid synovitis, remains unclear. Ligation of hyaluronan to CD44 upregulates Fas to induce apoptosis in RA synovial cell (Fujii 2001)
10. Stat3 is an attractive therapeutic target, as loss of Stat3 function led to exceptionally effective induction of apoptosis in RA synoviocytes (Krause 2002).

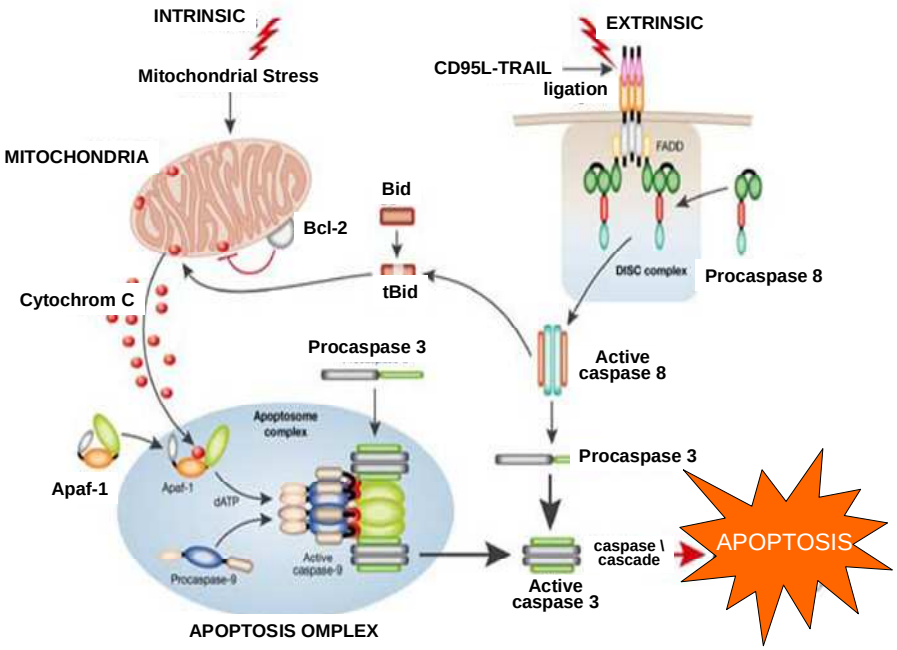

Figure 1. Pathways of Apoptosis potential for interventions. Death signals initiated by ligation of Fas-FasL, TNF-TNFR activate caspases for the proteolytic degradation of cytoskeletal, nuclear, DNA, cell cycle proteins and the activation of endonucleases. Two pathways are drawn in this pictorial, the mitochondrial pathway (intrinsic) and the death receptor pathway (extrinsic) (Perlman 2001b).

POTENTIAL INTERVENTION ON CELL CYCLE IN RA

Recently, a new class of cell cycle regulatory proteins were isolated, the cyclin-dependent kinase inhibitors (cdkI). These inhibitors bind to cdk or cdkcyclin complexes and inhibit kinase activity (Figure 1). The INK4 (inhibitors of cdk4) family of cdk-inhibitors includes p15, p16, p18 and p19, which bind to and inhibit only cdk4 and cdk6. The molecules belong to INK4 family can be a target treatment for RA. In contrast, the Cip/Kip (cdk2- interacting protein) family of cdk inhibitors (p21, p27 and p57) has a broad specificity for cdks. In response to a mitogenic signal, cyclin D/cdk4/6 binds to Cip/Kip family members, sequestering them from cyclin E/cdk2 complexes and thereby lowering the effective cdkI level. In the presence of the INK4 proteins, cyclin D/cdk4/6 activity is inhibited, freeing Cip/Kip family members that inactivate cyclin E/cdk2 and collectively induce arrest

in the G1 phase of the cell cycle. The removal of mitogens stops cyclin D synthesis, increasing the amount of Cip/Kip family members, and inhibiting cyclin E/cdk2 activity that leads to G1 arrest. These data demonstrate that the decision to progress through the cell cycle is regulated by the relative stoichiometric levels of cdk-cyclins and their cognate inhibitors**.** The molecules belong to INK4 family and Cip/Kip family members can be a target treatment for RA. To date, only a few investigations have focused on the cyclin dependent kinase inhibitors, in RA. Although p16 is associated with non-cycling cells, p16 expression was observed in RA. Immunoblot analysis of whole RA synovial tissue extracts revealed undetectable p21 and p16 expression, while p27 was observed, suggesting that the upregulation of p16 and p21 in cultured cells may be an *in vitro* phenomenon (Perlman 2001b). Mutation of *p53* gene may play a role in the decrease of apoptosis in RA. In experimental animal, *p53* suppression induces apoptosis through inhibition of Bax expression (Tak 2000; Yang 2005). While, inactivation of p53 blocks positive regulation of p21, similar effects to Bax and IL-6. In conclusion, p53 is contributory to synovial hyperplasia through p21 suppression (Perlman 2001b). Forced expression of a cyclin-dependent kinase inhibitor gene, $p21^{Cip1}$ in the synovial tissues was effective in treating animal models of rheumatoid arthritis (Nonomura 2003).

Table 1. Several molecules that play roles in apoptosis process (adapted from Catrina 2002; Ichikawa 2003; Perlman 2001a; Smith 2004; Tak 2000; Weng 2001; Yang 2004).

Pro-apoptotics	
Transcription Factors	*c-myc*, *c-fos*, NFκB,
Free radicals	Nitric oxide, oxygen radicals
Ligations	TNF-α, FasL
Bcl2 family	Bax, Bak, Bid, Bim, Mtd, Bik, Bok, Bip, Diva, Hrk, Bcl-xS
Efector molecules	Granzyme B
Anti-apoptotics	
Bcl2 family	Bcl-xL, Mcl-1, A1 dan Bcl-w
Protease Inhibitors	XIAP
Ligations	Soluble Fas
Others	Flip, IκB, TGFβ1, PTEN, CD44, wild type *p53*, PPARγ

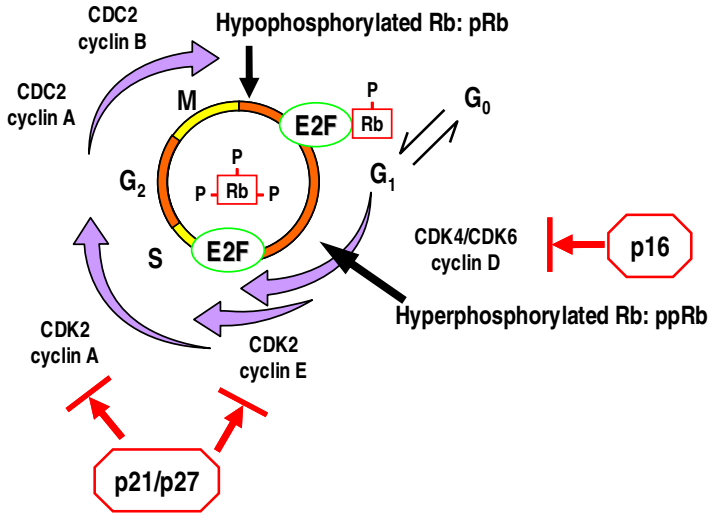

Figure 2. Molecules potential for target therapy. p16 inhibits cell cycle through supresión on cyclin D. p16 inhibits cell cycle through supresión on cyclin D cyclin A, while p 27 inhibits cell cycle through supresión on cyclin E.

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