Histol Histopathol (2006) 21: 205-211
DOI: 10.14670/HH-21.205

http://www.hh.um.es

Histology and Histopathology
Cellular and Molecular Biology

Review

Expression and function of cell cycle proteins in rheumatoid arthritis synovial tissue

E. Taranto and M. Leech
Centre for Inflammatory Diseases, Monash University Department of Medicine, Monash Medical Centre, Clayton, Melbourne, Australia

Summary. Rheumatoid Arthritis (RA) is a chronic disease characterised by synovial lining hyperplasia and progressive destruction of joint tissues. Experimental data suggests that abnormal alterations in the expression of proteins involved in maintaining homeostatic control of the cell cycle is involved in disease progression in RA. By contributing to the overgrowth of synovial tissue, factors such as dysregulated proliferation or reduced apoptosis of cells can directly influence the pathological outcome of RA.

Key words: Cell cycle, RA, Proliferation, Apoptosis, Arthritis

Introduction

Rheumatoid arthritis (RA) is the most common form of inflammatory arthritis causing severe disability and morbidity through progressive joint destruction. The aetiology of RA remains unknown. Early theories have implicated the involvement of autoantibodies and immune complexes. T-cell mediated antigen specific responses and T-cell independent cytokine networks have also been extensively investigated. Perhaps the most striking feature of RA is the excessive proliferation of the normal double cell layered synovium into a hyperplastic cellular aggregation and the formation of invading pannus tissue, comprised predominantly of macrophages and fibroblast-like synoviocytes (FLS). These cells actively invade and mediate the destruction of cartilage, peri-articular and subchondral bone. Growing evidence suggests that RA FLS found at the site of cartilage invasion, in the lining layer of the synovium, display distinct biological properties from both normal FLS and FLS derived from deeper layers of the RA synovium, distinguishing them as key players in the pathophysiology of RA.

Unlike normal fibroblasts which require adherence to a surface for growth, RA FLS are capable of growing in an anchorage-independent manner, permitting their growth into synovial fluid and contributing to the effects of inflammation in the joint (Lafyatis et al., 1989). Lafyatis et al. had additionally confirmed RA FLS to escape contact inhibition and grow beyond a monolayer. Muller-Ladner et al. (1996) showed that RA FLS, in contrast to FLS derived from osteoarthritic (OA) patients, were capable of autonomous invasion of human cartilage in an engrafted co-culture system in the severe combined immunodeficient (SCID) mouse. This finding demonstrates the capacity of RA FLS to maintain their proliferative and invasive molecular changes in the absence of external stimulation by inflammatory milieu.

By contributing to the overgrowth of synovial tissue, factors such as dysregulated growth or reduced apoptosis of cells are able to directly influence the pathological outcome of RA. The mammalian cell cycle is a tightly regulated process which governs the potential of a cell to undergo growth or apoptosis. Dysregulation of genes involved in maintaining homeostatic control of the cell cycle may be responsible for the altered growth observed in RA.

The capacity for cell cycle protein abnormalities to impact synovial overgrowth will be discussed in the following sections in terms of either a contribution to dysregulation of apoptosis or proliferative potential.

Dysregulated apoptosis

P53

p53 is at the head of key cellular pathways which integrate numerous signals regulating the cell cycle. These pathways become activated upon DNA damage, cellular stress or threat. This has been demonstrated by the rapid accumulation of p53 in DNA damage induced cells. Cells in a state of stress pose a potential threat to the organism as they are more likely to possess

Offprint requests to: Michelle Leech, Centre for Inflammatory Diseases, Monash University Department of Medicine, Monash Medical Centre, Locked Bag No 29, clayton, Melbourne, 3168 Australia. e-mail: michelle.leech@med.monash.edu.au

mutations and exhibit abnormal cell cycle control (Vogelstein et al., 2000). Thus in an attempt to contain damage caused by such cells, p53 functions as a 'brake' on cellular proliferation, blocking progression from G1 to S phase of the cell cycle and allowing time for repair before replication continues. This is accomplished through the transactivation of genes involved in cell cycle arrest such as p21. In more extreme circumstances where DNA damage is beyond repair, p53 induces cell death by apoptosis, transactivating members of the death receptor family including BAX and Fas.

It is evident from tumour biology research that functional inactivation of the p53 protein is a characteristic feature of most human cancers. In over 50% of these cases, functional inactivity it is known to be caused by mutations in the p53 gene (Hollstein et al., 1991)

The inadequate apoptosis noted in rheumatoid synovial tissue has been linked to dysfunctional p53. There have been some interesting experimental findings that have implicated the involvement of p53 in rheumatoid arthritis. Inactivation of endogenous p53 protein is demonstrated to result in enhanced RA FLS proliferation and increased invasiveness into cartilage extracts (Pap et al., 2000a). Moreover the characteristic phenotype of RA FLS is reported to be induced in normal FLS by inhibition of p53 (Aupperle et al., 1998). These studies have shown development of anchorage-independent growth, and impaired apoptosis in upon p53 inactivation.

Somatic mutations in p53 have been identified in RA FLS and synovial tissues in a number of studies (Firestein et al., 1997; Reme et al., 1998; Inazuka et al., 2000). Such mutations compromise p53 functionality and may therefore predispose to the hyper-proliferative phenotype of RA FLS. p53 mutations in isolation are not carcinogenic but may promote neoplastic transformation and furthermore are known to contribute to the pathogenesis of many neoplastic diseases. The presence of cells with transformed growth potential may contribute to the autonomous growth and invasion exhibited by inflamed synovial tissue.

Studies examining the expression of p53 protein in RA synovium are fraught with contention. While some describe elevated expression of p53 in RA, others suggest the contrary (Firestein et al., 1996, 1997; Sugiyama et al.,1996; Nickels et al., 1997; Reme et al., 1998; Taubert et al., 2000; Helmchen et al., 2005). Some workers have postulated that the increased p53 detected in some studies represents protective and appropriate upregulation of wt p53 in response to inflammatory stress. Others argue that increased p53 detected represents mutant p53 which is known to have a longer half-life than wild-type. Technical issues including RA heterogeneity, patient selection, regional variation and choice of antibody are also believed responsible for manifest discrepancies. Regardless of the precise reason for increased p53 detected in some studies, there is consensus that intact functioning p53 is desirable in intrinsic synovial cells to prevent hyperplastic responses to local inflammatory stress.

MDM2

p53 has additionally been shown to upregulate the murine double minute-2, (MDM2), gene which in turn inhibits the transcriptional activity of p53 and targets p53 protein for degradation via ubiquintination. This autoregulatory and bidirectional feedback between p53 and MDM2 is thought to allow cells to recover from p53-mediated G1 arrest (Freedman et al., 1999). Upon p53 activation the ability of MDM2 to down-regulate p53 is blocked allowing p53 levels to rise and exert effect (Momand et al., 2000).

In cancer biology, MDM2 has been proposed as a mechanism for loss of p53 function. In some tumors, both increased expression of MDM2 and mutations in the p53 gene are described suggesting that MDM2 may have other growth-promoting functions. In haemopoietic malignancy, MDM2 is ascribed a functional role in neoplasia, aggressiveness and resistance to anti-proliferative therapies. MDM2 has also been shown to bind Rb which is involved in blocking transcription factors associated with cell cycle progression, including E2F (Xiao et al., 1995; Hsieh et al., 1999). Mutation of Rb is also described in various forms of cancer (Kubota et al., 1995; Claudio et al 2000). When bound to Rb, MDM2 inhibits the ability of Rb to block E2F1 function, subsequently inhibiting G1 cell cycle arrest and permitting cell cycle progression (Xiao et al., 1995; Hsieh et al., 1999).

We have recently described increased MDM2 expression in RA synovial lining tissue and FLS (Taranto et al., 2005). Increased MDM2 has also recently been described in hemophilic synovitis (Hakobyan et al., 2004). The ability of MDM2 to interfere with p53 function and reduce p53 levels implicates it as one of many factors which could block p53 effects in RA synovium. Thus MDM2 may contribute to the hypoapoptotic phenotype and invasive potential of lining tissue. The capacity to down-regulate wt p53 levels in these regions may also have specific implications in terms of undermining the protective effect of wt p53 repression in MMP expression. Whether or not MDM2 can modulate the cell cycle in a p53 independent fashion remains to be seen.

PTEN

The protein phosphatase & tumour suppressor PTEN, (phosphatase and tensine homolog on chromosome ten), is involved in signal transduction and protection from tumorigenesis. Mutation and inactivation of PTEN is common in a variety of cancers and is associated with invasiveness and metastis (Li et al., 1997; Steck et al., 1997). PTEN plays an important role in growth regulation through its lipid phosphatase activity, which antagonises the effects of the PI3-kinase

pathway, thereby inhibiting downstream targets such as Akt involved in cell survival and proliferation (Stambolic et al., 1998). PTEN has additionally been shown to inhibit progression into S phase of the cell cycle by upregulating p27 (Hlobilkova et al., 2000). Others have shown that PTEN reduces expression of and nuclear localisation of cyclin D1, required for cell cycle progression, thus forcing cells into G1 phase cell cycle arrest (Radu et al., 2003).

Examination of PTEN in RA is limited to one study by Pap et al. (2000a,b). They found negligible expression in synovial lining tissues but abundant expression in sublining regions. By contrast, normal synovial tissue exhibited homogeneous expression of PTEN. In the same study only 40% of cultured RA FLS demonstrated expression of PTEN. Examination of RA FLS implanted with normal human cartilage into SCID mice exhibited minimal expression of PTEN. Interestingly, PTEN expression was not detected in those cells actively invading cartilage.

This data suggests that reduced PTEN expression may contribute to synovial invasion and hyperplasia through permissive effect. This is of particular importance given that PTEN is undetected in FLS found at the site of cartilage invasion (Pap et al., 2000b).

Trail

The tumour necrosis factor (TNF), family of cytokines are responsible for activating apoptosis via the death receptor pathway. Upon binding to their respective receptors, the TNF family cytokines including TNF-alpha, Fas-ligand (FasL) and TNF-related apoptosis-inducing ligand (TRAIL), act as extracellular activators of apoptosis. Upon engagement with TRAIL receptor 1 (DR4) or 2 (DR5), TRAIL is able to induce apoptosis through activation of the caspase cascade. TRAIL receptors 3 (DcR1) and 4 (DcR2) and the soluble TRAIL receptor osteoprotegerin all act as decoy receptors that block TRAIL mediated apoptosis (Simonet et al., 1997; Emery et al., 1998).

In a study examining the expression of TRAIL receptor in RA, it was found that DR5 was upregulated on RA FLS (Ichikawa et al., 2003). This finding correlated with an increased response to anti-DR5 antibody induced apoptosis. By contrast, synovial cells derived from OA patients did not express DR5 and were resistant to DR5 mediated apoptosis (Ichikawa et al., 2003). Interestingly, increased expression of DR5 is also a common feature of cancer (Walczak et al., 1999). In another study, neither DR4 nor DR5 could be detected in RA FLS, lymphocytes or macrophages (Pearlman et al., 2003). Ichikawa's findings are supported by recent work showing that syovial fluid fibroblasts derived from RA patients expressed DR5 that was functionally active in inducing apoptosis upon treatment with an agonistic anti-DR5 antibody (Miranda-Carus et al., 2004). This suggests that TRAIL receptors may not be expressed in a subset of RA patients.

In a murine xenograft model for human RA, treatment with anti DR5 antibodies was shown to significantly reduce the severity of bone and cartilage erosion (Ichikawa et al., 2003). Similarly, intraarticular adenoviral transfer of TRAIL in arthritic rabbit model was demonstrated to ameliorate disease (Yao et al., 2003). Conversely, immunoneutralisation of TRAIL in a collagen induced arthritis (CIA) model exacerbated arthritis (Song et al., 2000). In the same way TRAIL deficient mice demonstrate increased susceptibility to CIA (Lamhamedi-Cherradi et al., 2003).

Engagement of DR4 or DR5 has also been shown to have pro-proliferative properties via activation of JNK and NF-κB (Muhlenbeck et al., 2000). The extent of this capacity and its relevance to RA is yet to be examined. Targeting DR5 as a strategy for RA treatment may permit the inhibition of FLS mediated joint destruction. However, a wider population sample size should be examined to gauge the prevalence of DR5 receptor expression in RA patients.

Sentrin-1

Sentrin is a ubiquintin-like protein involved in regulating many key cellular processes. In a process similar to ubiquintination, sentrin functions at a post-translational level, modifying target proteins upon conjugation. This process is termed sentrinisation.

Increasing evidence suggests that sentrin controls pathways important for the surveillance of genome integrity including p53. It had been demonstrated that p53 undergoes sentrinisation at lysine 386 within the C-terminal region, the functional outcome of which has not been fully elucidated (Gostissa et al., 1999). Data shows that expression of SENP1, which cleaves sentrin from p53, subsequently results in p53 transactivation (Chen and Chen, 2003). Furthermore changing lysine 386 to arginine in p53 abolished p53 sentrinisation and correlated with an activation of p53 (Gostissa et al., 1999; Rodriguez et al., 1999). Sentrin may therefore play a role in the repression of p53. This has important implications in terms potential to undermine the effects of p53 in RA, especially because expression of sentrin-1 expression is described at the sight of cartilage invasion where dysfunctional p53 is ascribed a role in hyperplasia.

In RA synovial tissues, sentrin-1 mRNA is detected at significantly higher levels, especially at the site of cartilage invasion, compared to normal synovial tissues where sentrin can not be detected (Franz et al., 2000). RA FLS also exhibit increased sentrin mRNA expression compared to normal and OA FLS (Franz et al., 2000). In vivo, increased expression of sentrin-1 mRNA was detected in RA FLS coimplanted with normal human cartilage in the SCID mouse model (Franz et al., 2000). A functional role for sentrin in RA hyperplasia has been proposed by Franz et al. They suggest a protective effect from both anti-FAS/APO-1 and TNF induced cell death through the ability of sentrin to bind the cytoplasmic

domains of Fas/APO-1 and TNF receptor 1.

Increased proliferation and survival

Proto-oncogenes

Increased expression of various proto-oncogenes such as c-myc, erg-1 and ras have also been implicated as contributors to the aggressive and invasive behaviour of RA FLS. Hashiramoto et al. (1999), and colleagues have demonstrated that inhibition of c-myc in cultured RA FLS suppresses proliferation and can additionally induce apoptosis. FLS transfected with ras leads to the induction of cathepsin L involved in joint destruction and also shown to be up-regulated at sights of cartilage invasion (Joseph et al., 1987; Lemaire et al., 1994). Similarly, the co-localisation of MMPs with oncogenes in the lining layer is consistent with the view that proto-oncogenes are involved in their activation and effects (Okada et al., 1989; Lindy et al., 1997).

Stat

Signal transducer and activator of transcription (STAT) proteins are a family of cytoplasmic transcription factors that facilitate growth factor and cytokine induced signal transduction. Following cytokine stimulation and phosphorylation, STATs dimerise and translocate to the nucleus where they function to activate transcription.

STATS play a role in the signalling of proteins involved in regulating cell survival, proliferation, and differentiation. Dysregulation of STAT expression and function has been linked to many human diseases especially cancer and can contribute to cellular transformation.

Seven different STAT proteins (STAT 1-4, 5a, 5b and 6) have been identified. STAT-3 was the first STAT protein described in RA. Studies have demonstrated activated STAT-3 in synovial fluid and tissues derived from RA patients (Shouda et al., 2001), and shown that soluble factors present in RA synovial fluid, can activate STAT-3 in monocytes (Sengupta et al., 1995; Wang et al., 1995). STAT-3 activation was abolished following neutralisation of IL-6 (Sengupta et al., 1995). These results are in contrast to observations that STAT-1 and not STAT-3 is preferentially activated in RA and can also be blocked by IL-6 neutralisation (Yokota et al., 2001).

Over the years reports of increased expression of STAT-4, and 6 in RA have also emerged (Skapenko et al., 1999; Muller-Ladner et al., 2000). Despite numerous reports of STAT expression in RA, their involvement in synovitis remains unclear.

In a gene profile study examining the expression of STAT dependent genes in RA, increased expression of STAT-1 target genes was found, providing evidence that STAT-1 is functional in RA (van der Pouw Kraan et al., 2003) . In other cell types, STAT- 1 has been shown to be involved in the induction of growth arrest and apoptosis. In a study examining the effects of experimental arthritis in STAT-1-/- mice, STAT-1 deficiency was shown to exacerbate disease (De Hooge et al., 2004). These findings imply a protective role for STAT-1 in RA.

In a study utilising retroviral delivery of a dominant negative STAT-3 mutant that blocks STAT-3 function in synoviocytes, it was shown that STAT-3 deficiency significantly reduced cell growth and also resulted in spontaneous cell death (Krause et al., 2002). This observation suggests that STAT-3 contributes to synovial expansion in RA via suppression of synovial fibroblast apoptosis (Krause etal., 2002). This notion is supported by an earlier investigation in a collagen induced arthritis model where expression of a dominant-negative STAT-3 mutant was shown to reduce disease severity (Shouda et al., 2001). In accordance with these observations, sustained activation of STAT-3 has been shown to result in spontaneous development of RA-like arthritis in mice (Atsumi et al., 2002).

It seems the precise contribution of STATs to synovial hyperplasia in RA is difficult to determine as they are shown to have different and opposing properties that may vary with stage of disease.

p21

Phase transition of the cell cycle is dependent upon the activation and subsequent assembly of cyclin-dependent kinases (CDKs) and cyclins. Together, they function to induce cell cycle progression towards S phase and afterwards initiate mitosis in G2. The pro-proliferative activity of cyclin-CDK complexes are tightly regulated by CDK- inhibitors (CKIs) that function to block their catalytic activity. P21 is a member of the Cip/Kip family of inhibitors and is widely recognised for its role in induceing cell cycle arrest via its capacity to inhibit PCNA and cyclin A/E-CDK2 complexes.

In RA, it has been proposed that p21 activity in inducing arrest is reduced or deficient, thus contributing to synovial overgrowth. Studies have shown that levels of p21 protein in RA FLS and synovial tissues are negligible compared to OA (Pearlman et al., 2003). In some cases, p21 was not detectable in either OA or RA samples (Taniguchi et al., 1999). Over-expression of p21 in a collagen-induced arthritis model by adenoviral transfer was shown to inhibit FLS growth and additionally suppress the expression of key proinflammatory cytokines associated with RA, including IL-1ß, IL-6, and TNF-α (Nasu et al., 2000). In subsequent study, the same group showed overexpression of p21 could ameliorate rat adjuvant induced arthritis by inhibiting the growth of synovial fibroblasts (Nonomura et al., 2001). Moreover, Perlman et al., (2003) demonstrated that loss of p21 in synovial fibroblasts induces IL-6 and MMP production. Therefore in addition to dysfunctional cell cycle arrest, reduced p21 also contributes to RA pathology by inducing the expression of pro-inflammatory mediators.

In contrast a number of recent studies have suggested that p21 may have a permissive role in promoting cell cycle progression. Several studies demonstrate that p21 exists in complexes with active cyclin-CDKs. It has additionally been shown that cells deficient in p21 display a reduced capacity to complex cyclin-D1 and CDK4 suggesting p21 acts as an assembly factor promoting cyclin-D1-CDK4 binding thus contributing to cellular proliferation (Cheng et al., 1999). Moreover, LaBaer et al. (1997) demonstrated that p21 provides the localisation signal for cyclin-D1-CDK4 nuclear import. Loss of p21 is demonstrated to significantly reduce but not abolish the nuclear import of cylin D1 (Cheng et al., 1999). Further studies have shown that p21 promotes nuclear accumulation of cyclin-D1-CDK4 via its ability to inhibit nuclear export of cyclin D1 (Alt et al., 2002). Interestingly, Taniguchi et al. (1999) showed synoviocytes derived form RA patients expressed higher levels of p21 in response to cellular stress induced by radiation and serum starvation. This may represent a potential mechanism for enhanced synoviocyte survival in RA and a means by which stress induced cell cycle arrest contributes to the synovial expansion in RA.

Conclusion

RA FLS are pivitol players in the pathogenesis of joint destruction. The hyperplastic state of RA synovium is closely linked to invasion and destruction of joints. Similarly, resistance of RA FLS to apoptosis promotes synovial expansion and contributes to hyperplasia and joint destruction. There is a growing literature exploring the expression of regulatory and cell cycle genes in RA tissues and cells. Current evidence suggests that RA FLS have a distinct phenotype in relation to the expression of genes and proteins involved in cell cycle regulation.

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Accepted August 3, 2005