Commentary

p53 in rheumatoid arthritis: friend or foe?

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Received: 17 February 2000
Accepted: 15 March 2000
Published: 31 March 2000

Arthritis Res 2000, **2**:175–178

Abstract

The knowledge of transcription factors and proto-oncogenes has influenced the understanding of cell regulation, cell cycle, amd apoptotic cell death in rheumatoid arthritis (RA) synovium. In addition, the development of normal synovial fibroblasts into transformed-appearing aggressive synovial fibroblasts may be triggered by the lack of antiproliferative factors, such as p53, p53-associated molecules, other tumor suppressors, as well as by upregulation of anti-apoptotic genes. Therefore, data derived from experiments such as those performed by Tak and colleagues in this issue of *Arthritis Research* not only enrich the intensive discussion addressing the impact of *p53* on RA pathophysiology, they also may facilitate development of novel therapeutic approaches including *p53*-targeted gene therapy.

Keywords: gene therapy, MDM2, p53, p73, rheumatoid arthritis

Introduction

In this issue of *Arthritis Research* Tak *et al* [1] present interesting data on the development of apoptosis and expression of the tumour suppressor gene *p53* in adjuvant arthritis (AA). The intention of that study was to evaluate the AA model for its potential to test proapoptotic therapeutic strategies in RA.

Because those authors showed that expression of both apoptosis and p53 occurred rather late during the course of the disease, and as levels of p53 were even higher than in RA synovial tissue, the question arises as to whether p53 is the appropriate molecule to target. The tumour suppressor p53, in general, acts by inducing both growth arrest and apoptosis. On the basis of the results of that study [1], and of contributions to our current knowledge of the regulation of apoptosis in RA synovial fibroblasts [2–4], p53, when overexpressed early, may ameliorate the course of the disease. Before extrapolating this approach to human RA, however, the actual and/or potential role of p53 in the pathophysiology of RA needs to be discussed and defined. This issue is addressed by the following three questions.

p53 in cellular metabolism: gearing up or gearing down?

For more than a decade, p53 has been one of the most thoroughly examined genes in molecular tumor biology [5,6], which is illustrated by 16597 hits when 'p53' is entered into a PubMed search (http://www.ncbi.nlm.nih.gov/PubMed/). The physiologic 'tumor suppressor' function of p53 (ie its ability to induce apoptosis) is not restricted to a single pathway. Once it has formed a homotetramer [7], p53 binds to specific DNA sequences of various genes involved in control of cellular growth and apoptosis (eg those that encode $p21^{Waf1}$, bax, GADD, 14-3-3Δ, insulin-like growth factor-binding protein 3, caspases, Fas, the recently demonstrated KILLER/DR5 [8] and presumably numerous others).

AA = adjuvant arthritis; RA = rheumatoid arthritis.

Conversely, p53-modulated pathways that lead to cell arrest and apoptosis can also be activated by other molecules, such as members of the tumour necrosis factor family. One example is TRAIL, a molecule that is known to be involved specifically in the elimination of tumour cells [8,9].

The majority of these mechanisms is activated by damage to the cell and its genetic information, of which the latter needs to be stabilized for an extended period of time in order to secure the homeostasis of the organism. In general, activation of oncogenes, DNA damage, or exposure to oxidative stress is followed by activation of *p53*. As long as *p53* is not mutated (a deleterious effect that may occur during chronic inflammation [10]), activation of *p53* results in apoptosis or DNA repair, which means protection of the organism from development of continuous cellular proliferation and malignant transformation [11,12].

Apart from sole expression or absence of p53, its effect can also be modulated by cellular factors and infectious agents. For example, the oncogene MDM2 is critical for p53 subcellular distribution [13] and can degrade p53 rapidly [14], and human herpesvirus proteins are able to repress *p53* transcriptional activity [15], finally inducing malignant transformation.

Dysfunction of p53 occurs also when the *p53* gene itself is mutated; this knowledge, which was achieved more than a decade ago [16], is regarded as one of the basic scientific keys that facilitates understanding of the development of malignancies. The impact of *p53* gene mutations is reflected also by the fact that numerous tumours are based on *p53* mutations [17,18] (eg more than 70% of colorectal cancers bear mutations within the *p53* gene, which has already been targeted by gene therapy approaches [19]).

The p53 'picture' will soon become even more colourful, because recent data show that p53 is not a molecule that stands alone, but is a member of a growing family [20]. Currently the most important members of this family are p73 and Ket (named also p40, p51, p63 and p73L, respectively; for review see [21]).

In summary, when p53-dependent mechanisms are studied for novel therapeutic approaches, both sides of the p53 coin(s) need to be examined. Gearing up of p53 may induce apoptosis, and may downregulate inflammation as well as proliferation. On the other hand, gearing down of p53 may reveal key pathways that are involved in these mechanisms.

p53 in (rheumatoid) arthritis: what do we know?

When submitted to PubMed, the combination of p53 with arthritis elicits only 25 hits, and this number is even lower when 'rheumatoid' is added, resulting in only a few articles with the majority of the work performed by Firestein and various collaborators [1,3,10,22–29].

p53 is expressed in RA synovium, and cultured rheumatoid synovial fibroblasts probably contribute to this expression [22]. The idea that p53 may play an important role in regulation of cellular proliferation in rheumatoid synovium was supported by experiments that showed that, similar to in human tumours, transduction of synovial fibroblasts with papilloma virus E6 protein result in a decrease in p53 activity and increased cell growth [23]. Moreover, when examined in the severe combined immunodeficiency virus mouse model for RA [30], E6-transduced synovial fibroblasts showed enhanced aggressiveness towards co-implanted human cartilage [24].

Deducted from tumour biology as outlined above, p53 activity might also be altered when key bases in its gene sequence are mutated. Mutations at five 'hotspot' codons account for at least 20% of the known mutations [31]. Different research groups, however, have shown that, within rheumatoid synovium, various mutations apart from those in the known 'hot spots' are present, but are infrequent when compared with human tumours [25,32]. Of interest, variability of these mutations within different patient populations was high [25,32,33], some were dominant negative [26], and in some synovial fibroblast populations of RA patients living in the same geographic area they were even absent [27].

The experiments by Tak *et al* [1] contribute to this knowledge by demonstrating that expression of p53 in AA is not a feature that occurs very early during the course of the disease, but parallels increasing apoptosis in the inflamed synovial tissue. These data support immunohistological protein expression studies of p53 that have shown that expression of p53 in RA was considerably higher in RA than in osteoarthritis or reactive arthritis synovial specimens [28].

In addition, p53 appears to be involved not only in long-term disease, but also in early stages of human RA, because it could be detected shortly after clinical diagnosis was possible [28]. Therefore, it may be concluded that upregulation of p53 in AA is due to similar cell- and DNA-damaging mechanisms as outlined above, and that chronic inflammation in RA may also be a key stimulus for the upregulation of p53 and the number of apoptotic cells detected in RA synovium [10].

Furthermore, therapeutic approaches currently used for RA may also interfere with the expression of p53. For example, overexpression of tumour necrosis factor-α receptor p55 in RA synovial fibroblasts using adenovirus-based gene therapy results in upregulation of a MDM2-like

p53-binding protein (Fig. 1), which is presumably involved in regulation and cellular distribution of various members of the p53 family [13,14,34,35].

p53 in rheumatoid arthritis: what do we not know?

The various data obtained from the experiments that attempted to elucidate the role of p53 in (rheumatoid) arthritis clearly show that its presence must be associated with the course of the disease, and that there are still numerous questions to be answered before attributing a distinct role to p53 in the pathophysiology of RA.

First, we do not know whether p53 protein expression in rheumatoid synovium means expression of wild-type or mutated p53 protein, as all antibodies currently available for immunohistochemistry are not able to detect solely mutated p53.

Second, it needs to be clarified whether the (few and inconsistent) mutations in the *p53* gene sequence identified in synovial fibroblasts are sufficient to permit the generation of the transformed-appearing synovial fibroblasts located at the sites of invasion into cartilage and bone. In addition, mechanisms that counteract aberrant *p53* are presumably outweighing the antiapoptotic effects of mutated *p53*, because *p53* mutations (unlike in other tumours) do not result in synovial mesenchymal or lymphatic malignancies, for example sarcoma or 'SALT' (synovia-associated lymphatic tumour).

Third, it can still be speculated that p53 may not only be foe, but also a friend that helps to clear the synovium from aberrant cells induced by various stimuli that are genotoxic [10] and that inhibits matrix-degrading enzymes [36].

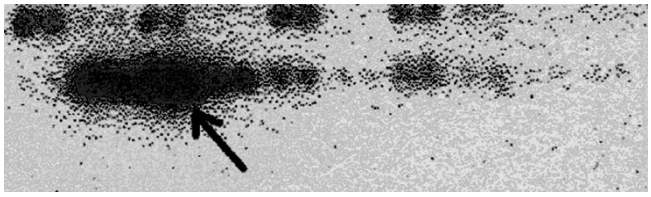
Fourth, it is most likely that p53 is only one of the potential antiproliferative molecules that are operative (or lacking) in rheumatoid synovium. This is illustrated by recent experiments that showed that the *p53* homologue *p73* [29] and the novel tumour suppressor *PTEN* [37] could only be detected in minute amounts in RA synovial fibroblasts.

Finally, it can be speculated that p53 is involved in susceptibility of a RA patient to antiproliferative medication (eg in tumours it is known not only that p53 inactivation results in upregulation of multidrug resistance genes [38], but also that mutations in the *p53* gene lead to acquired resistance to methotrexate [39]).

Conclusion

Inflammation, altered immune responses and synovial hyperplasia are typical features of RA [40]. There is increasing evidence that T-cell-independent pathways are key players in the progressive destruction of the affected joints. Knowledge of transcription factors and proto-oncogenes has influenced our understanding of cell regulation, cell cycle and apoptotic cell death, which can be summarized in an 'oncogene network' that is operative in RA synovial fibroblasts [41]. On the other hand, the development of normal synovial fibroblasts into transformed-appearing synovial fibroblasts that mediate joint destruction may not only be stimulated positively by upregulation of proto-oncogenes, but also by lack of antiproliferative genes such as *p53*, its relatives and other distinct tumour suppressors, as well as by upregulation of antiapoptotic genes [42]. Thus, data derived from experiments such as those performed by Tak *et al* [1] not only support our understanding of basic mechanisms that lead to this disabling disease, but also facilitate development of novel therapeutic strategies, including gene therapy targeting p53-dependent pathways [43,44].

Figure 1



Upregulation of MDM2-like p53 binding protein (arrow) following adenovirus-based TNFaRp55 gene transfer into rheumatoid synovial fibroblasts. (Analysis performed by Elena Neumann, University of Regensburg, Germany, using a Clontech Atlas® DNA array system. TNFaRp55 Ad5 generously provided by Dr John Mountz, University of Birmingham, AL, USA.)

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