A new defensive mechanism to prevent apoptosis in salivary ductal cells from patients with Sjögren’s syndrome: over-expression of p53 and p21

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Abstract

Objective. In Sjögren’s syndrome (SS), salivary acinar cells are destroyed even though ductal cells are frequently spared from destruction and can sometimes proliferate. We made the hypothesis that abnormalities of the tumour suppressor protein p53, either by mutations leading to proliferation or by activation of the functional wild-type p53, explain this phenomenon.

Methods. Immunohistochemistry to detect p53 and its transcription target p21, which is expressed only if p53 is functional and not mutated, was performed on labial salivary glands (LSG) from 10 patients with primary SS, all of whom had a Chisholm grade 4 LSG biopsy, and from 10 control patients with sicca symptoms or systemic diseases and a normal LSG biopsy (grade 0 or 1).

Results. The p53 antigen could be detected in ductal cells of nine of 10 LSG from SS patients and only one of 10 LSG from controls. The p21 antigen was detected in ductal cells of eight of 10 LSG from SS patients and two of 10 LSG from controls. The p53 and p21 antigens were localized in the same ductal cells in SS patients, and the positive ducts were those located around lymphoid foci.

Conclusion. The colocalization of p53 and its transcription factor p21 in salivary ductal cells surrounding lymphoid foci demonstrated that p53 was functional and not mutated. Its expression may be a defensive mechanism that provides ductal cells with time to repair DNA damage and prevents apoptosis. The lack of over-expression of p53 and p21 in acinar cells could be one of the key mechanisms of acinus destruction by apoptosis in SS and could be a target for new therapeutic strategies.

KEY WORDS: Sjögren’s disease, Apoptosis, p53, p21.

The p53 tumour suppressor protein, which has been called the ‘guardian of the genome’ [1], plays a central role in cell proliferation and death. Activation of p53 is induced by DNA damage or genotoxic stresses and leads to two alternative cellular responses: (i) arrest of the cell cycle mediated by its transcription target p21, allowing repair mechanisms or, in the presence of extensive DNA damage, (ii) induction of apoptosis through the expression of bax [1]. Loss of p53 activity allows the survival and proliferation of cells that would otherwise be eliminated. Accordingly, inactivation of the p53 tumour suppressor gene, whether through mutation, deletion or other mechanisms, is the most common genetic alteration in human cancers [2]. Paradoxically, most mutations induce an increase in the half-life of p53, leading to accumulation of the non-functional p53 protein, which becomes detectable by immunohistochemistry, whereas normal wild-type p53 is hardly detectable because of the short half-life of the protein. Therefore, the detection of p53 by immunohistochemical analysis may reflect either mutations of the p53 gene or the over-expression of the functional wild-type protein secondary to activation of the gene. To distinguish between these two mechanisms, it would be useful to detect the known transcriptional targets of the functional wild-type p53 protein, such as p21, which is the most likely factor to control cell cycle arrest.
Activation of the p53 gene has recently been associated with the pathogenesis of some autoimmune diseases, such as rheumatoid arthritis (RA) in humans [3–6] and Sjögren’s syndrome (SS) in humans [7] and in MRL/lpr mice [8]. Alternatively, mutations of the p53 gene that lead to impaired apoptosis may be involved in autoimmune diseases, for example in RA synovium, where they may mediate synovium proliferation [9, 10], or in non-Hodgkin lymphoma complicating SS [7].

SS is characterized by increased expression of the pro-apoptotic molecules Fas and Fas ligand in the three kinds of cell-type that are observed in labial salivary glands (LSG): lymphocytes, acinar cells and ductal cells [11]. However, only acinar cells are destroyed by apoptosis. Lymphocytes over-express Bcl2, which probably protects them from apoptosis [11]. Ductal cells are frequently spared from destruction and sometimes even proliferate, but the mechanism of this protection remains unknown. We made the hypothesis that this phenomenon can be explained by abnormalities of p53, arising either from mutations that lead to proliferation or by activation of the functional wild-type p53, acting as a defensive mechanism. To test this hypothesis, we looked for the expression of p53 and its transcriptional target p21 in LSG from patients with SS.

**FIG. 1.** Staining of epithelial cells of a salivary duct in an SS patient with an antibody directed against human p53. Indirect immunoperoxidase. F, focus of lymphocytes; A, acinus; D, duct. Magnification, ×400.

**FIG. 2.** Staining of epithelial cells of a salivary duct with an antibody directed against human p21. Adjacent paraffin section from the patient with SS shown in Fig. 1. Indirect immunoperoxidase. F, focus of lymphocytes; A, acinus; D, duct. Magnification, ×400.

**FIG. 3.** Absence of staining with an antibody directed against human p21. Adjacent paraffin section from the control subject shown in Fig. 1. Indirect immunoperoxidase. A, acinus; D, duct. Magnification, ×400.

**FIG. 4.** Absence of staining with an antibody directed against human p21. Adjacent paraffin section from the control subject shown in Fig. 3. Indirect immunoperoxidase. A, acinus; D, duct. Magnification, ×400.
Patients, material and methods

Patients

Ten patients fulfilling Californian [12] and revised European [13] criteria for primary SS, all of them with a Chisholm [14] grade 4 LSG biopsy, were included. None of the patients had lymphoma or other lymphoproliferative disease. None had been treated with immunosuppressive drugs. Ten patients with sicca symptoms or systemic diseases with a normal LSG biopsy (grade 0 or 1) were included as controls.

Immunohistochemistry

Paraffin sections 3 mm thick were deparaffinized and dehydrated in alcohol. For antigen retrieval, sections were pretreated in a microwave oven for 15 min at 450 W in citrate buffer, pH 7.36. The indirect immunoperoxidase method was used with purified mouse anti-human p53 protein (clone D07; Dako, Glostrup, Denmark) and purified mouse anti-human p21 protein (clone 6B6; Pharmingen, San Diego, CA, USA) as primary antibodies, at the dilutions of 1:50 and 1:100 respectively. Dilutions were optimized on sequential series of cutaneous and breast cancer sections known to be positive for these antibodies. Negative controls included omission of the primary antibody and use of an irrelevant antibody. Blinded evaluation was made by two pathologists (AJ and VM) with an Olympus AX70 microscope.

Results

By immunohistochemistry, p53 antigen could be detected in ductal cells from nine out of 10 LSG from SS patients but only one of 10 LSG from control subjects (Figs 1 and 3). Acinar cells were not positive in either series and a few lymphoid cells were stained in two of 10 LSG from SS patients and one of 10 LSG from controls.

The p21 antigen was detected in ductal cells from eight of 10 LSG from SS patients and two of 10 LSG from controls (Figs 2 and 4). Some acinar and lymphoid cells were positive in three SS patients.

The p53 and p21 antigens were localized in the same ductal cells in SS patients, and the positive ducts were those located around lymphoid foci. In the same areas, positively stained ducts and negatively stained acinar cells were observed (Figs 1 and 2).

Discussion

In LSG from 10 SS patients, we detected over-expression of p53 in ductal cells associated with over-expression of its transcription target p21, indicating that p53 was functional. Expression of p53 and p21 was present in ductal cells located around lymphoid infiltrates and was probably a defensive mechanism.

Impaired apoptosis is one of the key mechanisms in the pathogenesis of autoimmune diseases. Mutations of genes implicated in the apoptotic process, such as Fas and Fas ligand in lymphoid T-cells, occur in some murine models of autoimmune disorders and in some human autoimmune lymphoproliferative syndromes, and lead to increased survival of autoimmune lymphocytes [15]. On the other hand, Fas and Fas ligand may be over-expressed in target structures of autoimmune diseases, such as thyrocytes in autoimmune thyroiditis, the islets of Langerhans in diabetes and salivary epithelial cells in SS [11], leading to the destruction of epithelial cells by contact either with another epithelial cell (‘fratricide death’) or with a T-lymphocyte expressing Fas ligand.

In other locations, such as in RA synovium, the target cells of autoimmune diseases are not destroyed but proliferate. Acquired mutations of the p53 gene have been suspected to play a role in such states, as in cancer. Indeed, the p53 protein acts as a ‘checkpoint’ control in the cell cycle to permit the repair of damaged DNA by holding the cell in the G1 phase or by committing the cells to a pathway of apoptosis [1]. Although this remains controversial [5], various mutations of p53 have been detected in cultured synoviocytes from patients with RA and they could be one of the molecular events responsible for the proliferation of synoviocytes [6, 9, 10]. Likewise, p53 mutations in B lymphocytes could be one of the key mechanisms of lymphomagenesis in SS [7].

In SS that is not complicated by lymphoma, the absence of destruction of ductal cells, which may even proliferate, remains puzzling. The presence of p53 mutations might explain this phenomenon, and it could be argued that over-expression of the p53 protein supports this explanation. However, in a previous work, we could not detect any serum anti-p53 antibodies in 72 patients with SS that was not complicated by lymphoma [16], whereas serum anti-p53 antibodies are detected in 20–50% of patients with both cancer and p53 mutations. Moreover, Tapinos et al. [7] did not detect any p53 mutations in LSG from seven SS patients. In this study, we demonstrated colocalization of p53 and its transcription factor p21, which rules out the hypothesis of p53 mutations. Indeed, the expression of the p21 protein is mediated only by the wild-type functional p53 protein, not by its mutated form. The p21 protein may hold the cell in the G1 phase, allowing repair of damaged DNA and thus preventing apoptosis. Interestingly, p21 acts as a negative regulator of the proliferation of autoimmune T cells and female p21-deficient mice develop lupus-like autoimmune disease [17]. Therefore, over-expression of p53 on target tissues in autoimmune diseases is not always the result of mutation but can be related, in some cases, to the activation of the functional wild-type p53 protein in response to DNA attacks occurring as part of the autoimmune process, leading either to the arrest of the cell cycle in order to repair DNA or, if this is not possible, to apoptosis.
In conclusion, we have described a new defensive mechanism that provides ductal cells with enough time to repair DNA damage and thus prevents apoptosis. The colocalization of p53 and p21 in ducts surrounding the lymphoid foci is consistent with this defensive mechanism. Whether it is a mechanism that protects against primary dysfunction of epithelial cells—SS is often called autoimmune epithelitis—or a defensive phenomenon secondary to attacks on ducts and acinar cells by lymphoid infiltrates remains to be determined. Lastly, the absence of p53 and p21 over-expression in acinar cells could be one of the key mechanisms of acinus destruction by apoptosis in SS and could be a target for new therapeutic strategies.

References