CLINICAL AND ONCOLOGICAL SIGNIFICANCE OF ABERRANT FAS (APO-1/CD95) ISOFORM EXPRESSION IN ADULT T-CELL LEUKEMIA

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ABSTRACT

Fas (APO-1/CD95), a transmembrane death receptor mediating apoptosis, can induce cell death *in vivo* and *in vitro* of not only normal T-cells but also leukemic T-cells. This indicates that dysfunction in T-cell apoptosis may influence the natural history of the T-cell neoplasms, such as adult T-cell leukemia (ATL) caused by the retrovirus HTLV-1. Fas is ubiquitous, and down-regulated or mutated Fas has been widely detected in tumor cells that escape from elimination via Fas-mediated apoptosis. De novo fresh ATL cells and cell lines derived from the de novo cells, however, express Fas abundantly on the cell surface and are susceptible to Fas ligand and agonistic agents. On the other hand, there are two types of Fas gene transcripts, full-length and alternatively splicing truncated forms corresponding to membrane and soluble Fas isoforms, respectively. Focusing on membrane and soluble Fas isoforms and ATL pathology mediated by apoptosis, this paper reviews and discusses our ATL cases and ATL cell lines, which provide useful "experiments of nature" for understanding the role of Fas-mediated apoptosis in tumor biology.

KEY WORDS: Fas (APO-1/CD95), apoptosis, mutation, ATL, HTLV-1

INTRODUCTION

The Fas receptor, designated as APO-1 or CD95, is a 48-kD transmembrane glycoprotein belonging to the tumor necrosis factor receptor (TNFR) and nerve-growth superfamily, and has the potential action of mediating apoptotic signals after ligation with Fas ligand (FasL) or agonistic anti-Fas monoclonal antibodies (MoAb)(1-5). Fas is expressed in a variety of tissues and hematopoietic normal or leukemic cells. Therefore, Fas-mediated apoptosis has been suggested to play a crucial role not only in activated-induced cell death of normal T-cells but also probably in neoplastic cells which express Fas (6-7).

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The Fas gene found by Itoh and Nagata et al.(8) maps in chromosome 10q 24.1 and consists of 9 exons encoding extracellular (EC), transmembrane (TM), and intracytoplasmic (IC) domains. The Fas gene, moreover, encodes mainly two types of mRNA transcripts, full-length Fas mRNA, which is translated into membrane Fas isoform (mFas), which is in turn anchored on the cell-membrane by the TM domain, and alternatively spliced mRNA lacking exon 6 TMB&SFas mRNA, which is translated into soluble Fas isoform (sFas) which lacks the TM domain.(9, 10) Not only mFas but also sFas can provide protection from Fas-mediated apoptosis *in vivo* through the decoy mechanism, which interferes with ligation between mFas and FasL via a mechanism similar to that of soluble TN FR (11, 12).

Aberrant or defective mFas proteins caused by somatic mutation of the Fas gene or by an unknown control mechanism have been widely studied in relation to possible involvement in oncogenesis, evolution, and progression of tumours (13).

Especially, the lpr-mouse experimental model, in which the Fas gene was mutated by retroviral transposone insertion(14) encouraged such Fas clinical studies. Indeed, recent reports have supported such a theory, including those by Beltinger et al.(15) about acute lymphoblastic leukaemia (ALL), Landowski et al. (16) about myeloma, Tamiya et al.(17) about adult T-cell leukaemia (ATL), and von Reyher et al.(18) about colon carcinoma. However, most studies have examined the association between down-regulated or mutated mFas of neoplastic cells and tumour biology, because mFas is ubiquitous in a variety of cells (19).

On the other hand, up-regulation of mFas and unbalanced production of Fas isoforms have been noted in a few malignancies and some ATL cell lines, implying that not only mFas but also sFas is involved in apoptosis of tumour cells (20-21).

ATL is derived from an uncontrolled clonal expansion of T-cells, probably via transactivation mechanisms of pX gene products of human T-cell leukemia virus type-1 (HTLV-1) integrated into the genome. Like IL-2R, which is highly expressed on ATL cells due to transactivation by pX proteins (22, 23), mFas is also abundantly expressed (24, 25), however despite up-regulation of mFas, the cells can survive and form tumours which are associated with extremely poor prognosis. Recently, we have

encountered ATL cases with heterogeneous expression of mFas, varying from defective to markedly high, and with high serum levels of sFas(26). At present, little is known about neoplasms from the viewpoint of the membrane and soluble isoforms of Fas.

In this paper, we review clinical and oncological significance of quantitatively and qualitatively aberrant expression of the membrane and soluble isoforms of Fas in ATL on the basis of our recent studies published elsewhere (20, 25-27).

(I) Quantitatively aberrant Fas expression on ATL cells

ATL is well known to show a diverse clinical course and malignant behaviour, including cases of smoldering to acute and spontaneous regression of the tumor, suggesting apoptotic elimination of the tumor cells. Such a mechanism of eliminating tumor cells may be present in vivo, probably mediated through the mFas receptor, because ATL cells and ATL cell lines abundantly express mFas. If Fas function is significant in tumour biology, the expression levels of mFas isoform will affect the biological behaviour of the tumour cells in vivo, and will consequently affect the natural history of the clinical manifestation and prognosis. Here, since the sFas isoform probably acts

Fig. 1. Membrane (A) and soluble (B) Fas expression levels in each case among ATL cell line and ATL subtypes, which were subclassified into chronic, acute and lymphoma subtypes. Closed circles means ATL cases defective for mFas.

Fig. 2. Comparison of tumor burden and malignant behaviour according to mFas Status, positive (▨) or negative (■). Membrane Fas status, positive or negative, is not involved in tumor burden and in malignant behaviour; if anything, mFas-positive cases may have high tumor burden, but not significant.

Fig. 3. Comparison of tumor burden and malignant behaviour according to serum sFas status, high (▨) and low (■). Patients with sFas level, low column, show high tumor burden such as high ATL cell count, and bad malignant behaviour, that is, high LDH activity, high frequency of hypercalcemia and low rate of 3 year survival.

in vivo via its decoy function, the serum sFas level also should be considered to understand the Fas-mediated apoptosis in blood.

As shown in Fig. 1, ATL cells derived from about 90% of patients with ATL express not only mFas but also sFas at 2 to 5 fold higher density than normal controls, i.e. 3470 mFas molecules/cell and 1.54 ± 0.45 ng/ml sFas. ATL cell lines more abundantly express only mFas, with about 3 to 10 fold increased density, but are defective in sFas. These isoforms

can be measured using flow cytometric quantitative assays and enzyme linked immunosorbent assays. mFas expressed by malignant cells is actually functional at least in vitro after triggering by agonistic anti-Fas MoAb and recombinant FasL (26). This means that ATL cells expressing mFas are in a primed state in vivo for apoptosis via Fas, which is probably important for tumor manifestation.

On the other hand, about 10% of ATL patients have no detectable mFas on the cell surface, but

have a high sFas level in their sera, with no significant correlation between the mFas and sFas levels. Either mFas or sFas, and frequently both, in ATL cells are usually up-regulated and have the potential to receive the signal from the ligand.

(II) sFas level, but not mFas level, is related to clinical tumor biology.

The findings about aberrant Fas isoform expression described above suggest that high tumor burden such as reflected by large lymph node (LN) and high count of ATL cells in blood and highly malignant behaviour manifested by serum lactate dehydrogenase (LDH) activity, hypercalcemia and poor prognosis of 3-year survival rate (3y SR), is not related to mFas, as shown in Fig.2. In contrast, as shown in Fig. 3, a high sFas level is apparently associated with high tumor burden and poor malignant behaviour, especially an extremely short survival time, suggesting the decoy function of the sFas in blood. In ATL pathology, sFas thus appears to be more important than mFas in determining clinical aspects of the tumor, whereas the current opinion about solid tumors is that mFas is important.

More recently, there have been interesting studies that were instructive about the relationship between Fas isoforms and tumor biology. In these

studies, Natali et al.(28) and Beletskaya et al.(29) reported that the presence of alternative splicing isoforms, such as Fas with deleted TM, affected the cell resistance to Fas-mediated apoptosis, and that these isoforms were potentially able to neutralize anti-Fas or FasL.

The question then arises whether mFas status in ATL cells is flexible or not, and of the role of mFas in ATL biology. To understand these issues, it is convenient to examine our mFas-negative ATL cells. First of all, it has been demonstrated that the ATL cells preserve normally both full-length and truncated Fas mRNAs, corresponding to mFas and sFas receptors (20,25,26). Indeed, de novo fresh mFas-negative ATL cells come to express detectable mFas along with the change to dominant expression of the full-length mRNA and decrease of the truncated mRNA (Fig. 4). A similar phenomenon has also been reported in B-chronic lymphoid leukemia (CLL) cells, in which mFas is deficient or defective. Fresh CLL cells, which express low levels of mFas, are resistant to agonistic anti-Fas MoAb-mediated apoptosis (30). After culturing cells in the presence of the mitogens, resulting in mFas expression on the cell surface, the cells become susceptible to agonistic anti-Fas.

On the other hand, there was a report that Fas-negative ATL cells from a patient were caused by

Fig. 4. Changes of mFas density (A and B) and mRNA (C) levels before and after short term cultures with PWA in a patient with mFas negative ATL

aberrant transcripts resulting in the premature termination of both alleles and consequently the loss of expression of mFas(17).

In any case, our observations from many ATL cases indicate that there exists some dys-regulation in the mechanism of control of the balance between

Fig. 5. Sequence analysis of Fas cDNA derived from KOB ATL cell line.

Fig. 6. Expected structure of truncated Fas receptor in the KOB ATL cell line. The truncated Fas antigen lacks 20-bp at exon 9. The frame shift caused by the deletion generates a premature stop codon at amino acid 239.

Fig. 7. Morphological changes after treatment with agonistic anti-Fas MoAb in wild type Jurkat, LXSJ (vector only), and LdelSN (vector-the mutant Fas). Jurkat cell line cells.

Fig. 8. A possible mechanism of dominant negative interference in the mutant truncated Fas transfected-Jurkat T-cell line, which has wild-type mFas.

the two isoforms, membrane or soluble, and that, consequently, overproduction of sFas is involved in the natural history of ATL.

(III) Mutated Fas in ATL

At first, mutation in Fas-encoding gene was reported in *lpr* and *lpr^g* mice, which are models of auto-immune disease. These mice fail to eliminate self-reactive T-lymphocytes due to the mutation of Fas gene, resulting in the accumulation of a large number of non-malignant T-lymphocytes in the lymph nodes and spleen(6). Recently, nucleotide sequence analysis of Fas transcripts has revealed the mutations probably resulting in congenital disorders in humans such as auto-immune lymphoproliferative syndrome (ALPS)(31) and Canale-Smith syndrome(32), resembling the *lpr* phenotype in mice with mutated Fas. These patients have defective Fas-mediated T-cell apoptosis associated with unique Fas gene mutation. More recently, even in acquired neoplasms such as myeloma, ALL, colon cancer, and hepatoma, mutations of Fas have also been demonstrated, implying that the tumorigenesis may be due to mutations of Fas. The membrane Fas status on these tumor cells varied from complete lack or deficiency to normal density. In some cases, although there was mFas lacking the intracytoplasmic signaling domain, no cell death could be induced through the Fas-signal system(33). Such cases, however, are rare among the patients examined for Fas mutation(15-18) so that the remaining majority of cases should be considered to have normal Fas genes.

Now, there have been two reports about Fas mutations in ATL; one by Tamiya et al.(17) and the other by our group. Our study showed a dominant

negative defect in Fas-induced apoptosis in an ATL cell line, KOB, and in the cell from which KOB was derived, de novo fresh ATL cells from a patient with ATL(27). The cells were shown by flow cytometry to express mFas, although they showed resistance to induction of cell death by agonistic anti-Fas MoAb. Molecular analysis of the PCR products from not only the cell line but also the original de novo cells showed a 20-bp deletion in exon 9 in the mutated Fas mRNA, resulting in a frameshift and the generation of a premature stop codon at amino acid 239, as shown in Fig. 5 and 6. A retrovirus-mediated gene transfer of the truncated Fas into Jurkat cells susceptible to Fas-mediated apoptosis rendered the cells resistant to the apoptosis. Such a mechanism is based on a dominant negative interference because of the effect of the endogenous wild-type Fas by the transfected mutant Fas (Fig. 7 and 8). More interestingly, in this case ATL cells at the onset had the wild-type Fas gene in the lymph node and later an ATL subclone acquired a Fas mutation in the crisis phase leading to malignant behaviour, enabling the subclone to escape from apoptosis mediated by the Fas signalling system and proliferate in the body.

(IV) Conclusion

De novo fresh ATL cells and ATL cell lines heterogeneously express mFas and sFas. Aberrant Fas isoform expression in ATL cells is instructive about the isoform roles in vivo and the significance of the isoforms in clinical oncology. Using these convenient "experiments of nature", further aspects of the complex relationship between tumorigenesis and Fas-mediated apoptosis regulated by various genes and their products will be elucidated in the near future.

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