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Caspase-mediated cleavage of X-ray repair cross-complementing group 4 promotes apoptosis by enhancing nuclear translocation of caspase-activated DNase

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ABSTRACT

X-ray repair cross-complementing group 4 (XRCC4), a repair protein for DNA double-strand breaks, is cleaved by caspases during apoptosis. In this study, we examined the role of XRCC4 in apoptosis. Cell lines, derived from XRCC4-deficient M10 mouse lymphoma cells and stably expressing wild-type XRCC4 or caspase-resistant XRCC4, were established and treated with staurosporine (STS) to induce apoptosis. In STS-induced apoptosis, expression of wild-type, but not caspase-resistant, XRCC4 in XRCC4-deficient cells enhanced oligonucleosomal DNA fragmentation and the appearance of TUNEL-positive cells by promoting nuclear translocation of caspase-activated DNase (CAD), a major nuclease for oligonucleosomal DNA fragmentation. CAD activity is reportedly regulated by the ratio of two inhibitor of CAD (ICAD) splice variants, ICAD-L and ICAD-S mRNA, which, respectively, produce proteins with and without the ability to transport CAD into the nucleus. The XRCC4-dependent promotion of nuclear import of CAD in STS-treated cells was associated with reduction of ICAD-S mRNA and protein, and enhancement of phosphorylation and nuclear import of serine/arginine-rich splicing factor (SRSF) 1. These XRCC4-dependent, apoptosis-enhancing effects were canceled by depletion of SRSF1 or SR protein kinase (SRPK) 1. In addition, overexpression of SRSF1 in XRCC4-deficient cells restored the normal level of apoptosis, suggesting that SRSF1 functions downstream of XRCC4 in activating CAD. This XRCC4-dependent, SRPK1/SRSF1-mediated regulatory mechanism was conserved in apoptosis in Jurkat human leukemia cells triggered by STS, and by two widely used anti-cancer agents, Paclitaxel and Vincristine. These data imply that the level of XRCC4 expression could be used to predict the effects of apoptosis-inducing drugs in cancer treatment.

1. Introduction

Apoptosis is a mechanism by which cells undergo programmed cell death in response to external or internal signals. Various anti-cancer drugs induce apoptosis, and dysregulation of apoptotic pathways in cancer cells leads not only to chemotherapy resistance, but also to further tumor development. A number of the pre-mRNAs of genes involved in apoptotic pathways are alternatively spliced, and the subsequent protein isoforms often possess opposite functions in apoptosis. For example, the B-cell lymphoma (Bcl)-2 family gene *bcl-x* produces two protein isoforms, Bcl-x_L and Bcl-x_S, with anti- and pro-apoptotic functions, respectively [1]. Production of apoptotic factor isoforms by alternative splicing allows for fine-tuning the regulation of life-or-death decisions by cells.

Cleavage of nuclear DNA into oligonucleosomal DNA fragments occurs specifically during apoptotic cell death. Caspase-activated DNase

Abbreviations: XRCC4, X-ray repair cross-complementing group 4; STS, staurosporine; CAD, caspase-activated DNase; ICAD, inhibitor of CAD; SRSF, serine/arginine-rich splicing factor; SRPK, SR protein kinase; Bcl, B-cell lymphoma; CLK, Cdc2-like kinase; Lig4, DNA ligase IV; NHEJ, non-homologous end joining; DSBs, DNA double-strand breaks; PTX, paclitaxel; VCR, vincristine; z-VAD-fmk, benzyloxycarbonyl-Val-Ala-Asp (OMe) fluoromethylketone; DMSO, dimethyl sulfoxide; RT-qPCR, RT-quantitative PCR; NLS, nuclear localization signal

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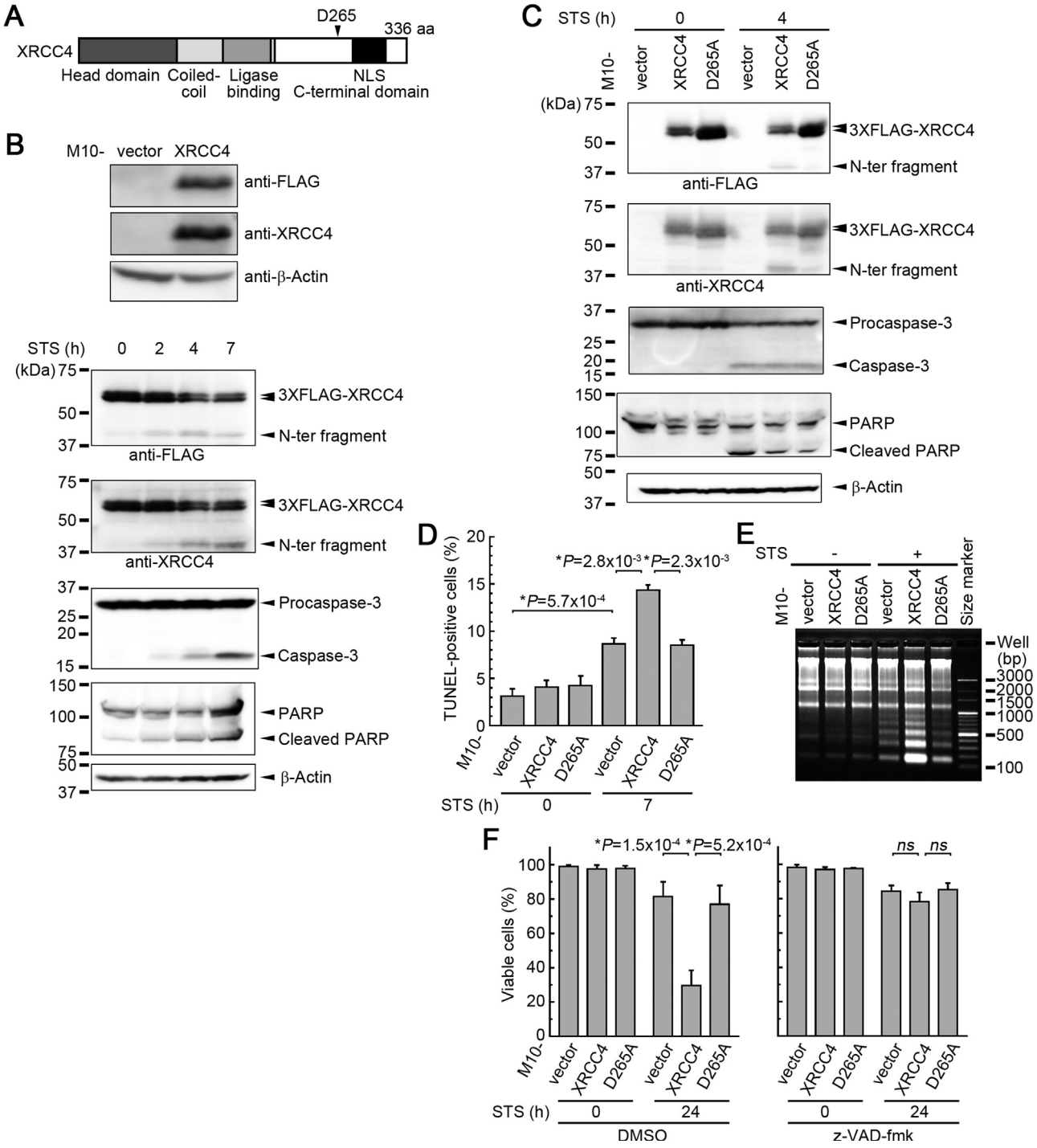

Fig. 1. Caspase-mediated cleavage of XRCC4 enhances apoptosis. (A) Diagram of the structure of XRCC4 protein. The location of an aspartic acid residue, a target for caspase-3/7 [25], is shown with an arrowhead. NLS, nuclear localization signal [36]. (B) Lysates of M10-vector and M10-XRCC4 cells were subjected to western blotting analyses with the indicated antibodies (upper panel). M10-XRCC4 cells were treated with STS, lysed at the indicated times, and the lysates were subjected to western blotting analyses with antibodies against the indicated proteins (lower panel). (C, D) Three cell lines were treated with STS for the indicated times, and cells were then subjected to western blotting analyses with antibodies against the indicated proteins (C), and to a TUNEL assay (D). (E) Three cell lines were incubated with (+) or without (-) STS for 7 h, and cells were then subjected to a DNA laddering assay. (F) Three cell lines were treated with STS for the indicated times with or without z-VAD-fmk. The percentage of viable cells was assessed by trypan blue dye exclusion assay.

(CAD) plays a major role in oligonucleosomal DNA fragmentation [2–5]. CAD forms a protein complex with the inhibitor of CAD (ICAD) [2,4,6], and the two ICAD mRNA variants ICAD-S and ICAD-L are generated by alternative splicing of ICAD pre-mRNA [7]. CAD activity is regulated by caspase-dependent degradation of ICAD and nuclear import of CAD [2,4,6]. Although both ICAD-L and ICAD-S bind CAD and inhibit CAD activity [6,8], only ICAD-L possesses chaperone activity for correct folding of CAD and a nuclear localization signal to help nuclear import of CAD [4,6,9–11].

Serine- and arginine-rich splicing factor 1 (SRSF1), a prototypic splicing factor in the SR protein family, has been shown to regulate the alternative splicing of ICAD pre-mRNA [12,13]. SRSF1 pre-mRNA is alternatively spliced to produce a major mRNA variant (SRSF1-L), which generates the canonical SRSF1, and several mRNA variants that are non-translated or degraded by the nonsense-mediated mRNA decay pathway [14]. SR proteins contain an arginine-serine-rich motif (the RS