Tumor-induced Apoptosis of T Cells: Amplification by a Mitochondrial Cascade

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Abstract

We have recently reported that apoptosis of T cells induced by squamous cell carcinoma of the head and neck (SCCHN) is partly Fas dependent. This tumor-induced T-cell death is mediated by the activities of caspase-8 and caspase-3 and is partially inhibited by antibodies to either Fas or Fas ligand. We report here that in contrast to apoptosis induced by agonistic anti-Fas antibody (Ab), the tumor-induced apoptotic cascade in Jurkat cells is significantly amplified by a mitochondrial loop. The involvement of mitochondria in tumor-induced apoptosis of T cells was demonstrated by changes in mitochondrial permeability transition as assessed by 3,3'-dihexylxocarboxyfluorescein staining, by cleavage of cytochrome c to the cytosol, and by the presence of active subunits of caspase-9 in Jurkat T cells cocultured with tumor cells. To further elucidate the significance of mitochondria in tumor-induced T-cell death, we investigated the effects of various inhibitors of the mitochondrial pathway. Specific antioxidants, as well as two inhibitors of mitochondria permeability transition, bongkrekic acid and cyclosporin A, significantly blocked the DNA degradation induced in Jurkat T cells by SCCHN cells. However, these inhibitors had no effect on cells triggered by anti-Fas Ab. Furthermore, a cell-permeable inhibitor of caspase-9, Ac-LEHD-CHO, which did not inhibit T-cell apoptosis induced by anti-Fas Ab, markedly inhibited apoptosis induced by etoposide or by coculture of Jurkat with SCCHN cells. These findings demonstrate that apoptotic cascades induced in Jurkat T lymphocytes by anti-Fas Ab or tumor cells are differentially susceptible to a panel of inhibitors of mitochondrial apoptotic events. It appears that besides the Fas-mediated pathway, additional mitochondria-dependent cascades are involved in apoptosis of tumor-associated lymphocytes. Inhibition of mitochondria-dependent cascades of caspase activation should be considered to enhance the success of immunotherapy or vaccination protocols in cancer.

Introduction

Recent studies suggest that human carcinoma cells of various origins can activate intrinsic programmed cell death in lymphocytes interacting with the tumor in situ and in vitro (1–3). This tumor-induced apoptosis of lymphocytes may have important implications for the success of therapeutic regimens, including vaccination strategies (4). Because tumor-induced apoptosis of lymphocytes may be mediated by an array of death receptors coexpressed on T cells or by tumor-derived soluble factors, it is important to characterize those intracellular events that may be potential targets for therapeutic intervention to minimize T-cell apoptosis. The caspases, a family of cysteine proteases, play critical roles in the execution phase of apoptosis and are responsible for many of the biochemical and morphological changes associated with apoptosis (5–7). Caspase-8 has been identified as the most apical caspase in apoptosis induced by several death receptors, including Fas and TNFR1 (8). Fas-associated death domain is recruited directly to ligated Fas or indirectly to ligated TNFR1, resulting in recruitment and autoactivation of caspase-8. Active caspase-8 cleaves and activates downstream caspases, initiating the caspase cascade. Caspase-9 has been proposed as the initiating caspase in a pathway of apoptosis that is death receptor independent (9, 10). In the presence of dATP and cytochrome c, the long N-H2-terminal domain of caspase-9 interacts with APAF-1, resulting in activation of caspase-9. Active caspase-9 can then activate the effector caspase-3, -6, and -7 (10, 11). Thus, there are at least two major mechanisms by which a caspase cascade may be initiated: (a) one involving caspase-8; and (b) the other involving caspase-9 as the most apical caspase.

These two basic pathways of caspase activation allow predictions as to how the apoptotic cascade is regulated under different circumstances. It is expected that various inhibitors of apoptosis, including Bcl-2 family members, CrmA, Flice-inhibitory protein, or inhibitors of apoptosis, which target different caspases or intracellular apoptotic events, will differentially regulate the two caspase activation cascades. For example, antiapoptotic Bcl-2 family members bind to mitochondria and inhibit release of cytochrome c (12, 13). Therefore, apoptotic signaling via death receptors should be resistant to Bcl-2 (14). However, it seems that Bcl-2 and Bcl-xL can also interfere with Fas-mediated apoptosis in cells in which the Fas/Fas-associated death domain/pro-caspase-8 recruitment is not efficient (15). Because Fas ligation is associated with release of cytochrome c, it raises the possibility of cross-talk between the two basic pathways. Recently a mechanism of cross-talk between caspase-8 and caspase-9 via mitochondria was identified (9, 16, 17). BID, a proapoptotic member of the Bcl-2 family, is cleaved by caspase-8, and its C-OH-terminal fragment translocates to the mitochondria and triggers release of cytochrome c (9, 16, 17). Depletion of BID from cytosolic extracts disrupts the ability of caspase-8 to trigger cytochrome c release in vitro (17).

The current study investigated intracellular apoptotic events in Jurkat T cells interacting with SCCHN. The intracellular effector molecules involved in execution of tumor-induced death of lymphocytes, which might serve as potential targets for inhibition of apoptosis, have not yet been elucidated. Our recent studies (18, 19) demonstrated that apoptosis induced in T lymphocytes by tumor cells was in part, Fas mediated and involved activation of caspase-8 and -9.

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3 The abbreviations used are: TNFR, tumor necrosis factor receptor; BA, bongkrekic acid; CsA, cyclosporin A; dThd, dThd, deoxythymidine; HM, heavy membrane; PT, permeability transition; ANT, adenine nucleotide translocator.
In the present study, we investigated the role of a mitochondrial cascade and its significance in SCCHN-induced apoptosis of Jurkat T lymphocytes. Our findings suggest that in contrast to Fas-mediated apoptosis of Jurkat cells, which is mitochondria independent, mitochondria have a significant effector role in tumor-induced cell death of interacting T cells.

Materials and Methods

Reagents. Agonistic anti-Fas Ab (CH-11; IgM) was purchased from Upstate Biotechnology (Lake Placid, NY), rabbit anti-caspase-9 Ab (clone H-83) was from Santa Cruz Biotechnology (Santa Cruz, CA), anti-cytochrome c was from PharMingen (San Diego, CA), and anti-cytochrome c oxidase was from Molecular Probes (Eugene, OR). Rabbit anti-BID polyclonal Ab used has been described previously (20). The caspase-9 inhibitor, Ac-LEHD.CHO, was purchased from Novabiochem (San Diego, CA). DiOC(6) and CMXRos were from Molecular Probes, and GAMIG-conjugated magnetic beads were from PerSeptive Diagnostics (Cambridge, MA). Staurosporin, etoposide (VP-16), CsA, diamide, and the antioxidants DPI and NAC were purchased from Sigma (St. Louis, MO). BA was a generous gift from Dr. J. A. Duine (University of Delft, Delft, the Netherlands). Anti-α6β1 mAb (A9) was a generous gift from Dr. T. E. Carey (University of Michigan Cancer Center, Ann Arbor, MI).

Cell Lines. The human Jurkat T-cell leukemia cell line was grown in RPMI 1640 supplemented with 10% FCS, 50 mM HEPES buffer, and 2 mM l-glutamine (Life Technologies, Inc.). This Jurkat cell line (Ju-S) is sensitive to a variety of apoptosis-inducing agents, including anti-Fas Ab, VP-16, and staurosporin. A Jurkat cell line (Ju-R) resistant to apoptosis induced by either anti-Fas Ab or VP-16 was used as a negative control (3). The previously described SCCHN cell lines PCI-13, PCI-52, OSC-19, SCC-68, and SCC-74 were grown in MEM supplemented with 10% FCS, 50 mM HEPES buffer, and 2 mM l-glutamine (4, 18, 19).

Lymphocytes and Tumor Coculture. To induce apoptosis or apoptosis-related changes in lymphocytes, SCCHN cell lines were cocultured with Jurkat cells for 16–24 h at a tumor:lymphocyte cell ratio ranging from 20:1 to 80:1. To assess processing of intracellular proteins, Jurkat cells were negatively selected by removal of SCCHN cells using epithelial-specific α6β1 mAb (A9) and GAMIG-conjugated magnetic beads. To this end, cocultures of SCCHN and Jurkat cells were incubated with A9 mAb at 50 μg/ml cells/ml on ice for 1 h. The cells were washed three times in cold medium and subjected to two cycles of incubation with GAMIG-coated magnetic beads (30:1; beads: cell ratio). As assessed by flow cytometry, tumor cells were efficiently removed because 99% of the negatively selected cells did not bind anti-α6β1 integrin mAb. Jurkat cells cocultured with normal skin fibroblasts or triggered by agonistic anti-Fas Ab (CH-11; 200 ng/ml), staurosporin (0.5 μM), or VP-16 (20 μM) served as controls. Inhibitors of apoptosis, including BA, CsA, caspase-9 inhibitors, or antioxidants, were added to Jurkat cells 2 h before the induction of apoptosis. Stock solutions of drugs in DMSO were stored at −20°C. Control cells received solvent alone. The final concentration of DMSO solvent in the culture medium never exceeded 1% (v/v), which was nontoxic to the cells.

Analysis of Apoptosis. DNA fragmentation was assessed by the JAM assay, in which loss of [3H]TdR-labeled DNA was measured (3). DNA labeling of Jurkat target cells was performed by incubation of the cells in the presence of 5 μCi/ml [3H]TdR for 18–24 h at 37°C. Tumor cells were cocultured with [3H]TdR-labeled target cells for 16 h at 37°C to tumor:lymphocyte or normal fibroblast:lymphocyte cell ratios ranging from 10:1 to 80:1. At the end of the coculture period, the cells were harvested (Mach IIM; TOMTEC) onto glass fiber filters. The radioactivity of unfragmented DNA retained on the glass fiber filters was measured by liquid scintillation counting. Specific DNA fragmentation was calculated according to the following formula: percentage of specific DNA fragmentation = 100 × (S − E)/S, where S represents retained DNA in the absence of effector cells (spontaneous), and E represents experimentally retained DNA in the presence of tumor (effector) cells.

Analysis of Protein Expression by Western Blotting. Jurkat cells, either controls or those treated with staurosporin or anti-Fas Ab or coinoculated with tumor cells, were stained with CMXRos (200 nm) for 30 min at 37°C. After washing the cells with PBS, the cytopsins of the cells were prepared and fixed in 2% paraformaldehyde for 10 min at room temperature. The cells were then washed five times in PBS. After permeabilization by 0.1% Triton X-100 in PBS at 4°C for 15 min, the cells were washed three times with PBS and three times with 0.5% BSA and 0.15% glycine (buffer A). The cytopsins were then treated with a 4% dilution of goat serum in buffer A for 1 h at room temperature. After five washes in buffer A, the cells were treated with rabbit anti-BID and mouse anti-CD3 at 4°C overnight. After five additional washes in PBS/BSA, FITC-conjugated goat antirabbit Ab and biotin-conjugated goat antimouse Ab were added for 1 h at room temperature. After five washes, cytopsins were treated with Cy5-streptavidin for 1 h, followed by nuclei staining with Hoechst (1 μg/ml) and mounted using Gelvatol (Monsanto, St. Louis, MO). The cytopsins were then observed by fluorescence microscopy using a Zeiss Axiosvert 135 microscope equipped with a Hamamatsu Orca camera and filter sets for Hoescht, FITC, rhodamine (to detect CMXRos), and Cy5. Images were collected using Meta Morph (Universal Imaging Corp.).

Results

Mitochondria Involvement in Tumor-induced Apoptosis of T Cells. To assess the role of mitochondria in tumor-induced apoptosis of T cells, we first analyzed changes in mitochondria pore PT using the mitochondria dye DiOC(6)(3). After coinoculation with tumor cells, CD3+ Jurkat cells were assessed for loss of DiOC(6)(3) uptake, which is indicative of altered pore PT. Changes in the PT were induced in Jurkat cells during a 16-h coculture with SCCHN cells, including PCI-13 (Fig. 1), PCI-52, OSC-19, SCC-68, or SCC-74 (data not shown). No changes were detected in DiOC(6)(3) staining in Jurkat cells cocultured with control fibroblasts (data not shown).

Cleavage of BID and Translocation to the Mitochondria in T Cells Interacting with Tumor Cells. BID, a BH3 domain-containing Bcl-2 family member, has recently been identified as a factor that relays signals from cell surface death receptors to the mitochondria (16, 17, 23). A COOH-terminal fragment of BID cleaved by caspase

8 translocates to the mitochondria and triggers cytochrome c release (17). To investigate the involvement of BID in tumor-induced apoptosis of lymphocytes, the presence of the BID proform or its cleaved fragments was examined by Western blotting. As shown in Fig. 2, BID was cleaved in apoptosis-sensitive (Ju-S) but not in apoptosis-resistant (Ju-R) Jurkat cells cocultured with tumor cells. In Jurkat cells treated with agonistic anti-Fas Ab, most of the BID proform (M, 25,000) was processed, and two cleaved fragments of M, 13,000 and M, 15,000 were detected. In Jurkat cells coincubated with tumor cells, the BID proform was partly processed, and only one cleavage fragment was detected. The difference in BID processing may relate to the differential apoptotic signal delivered by agonistic Fas Ab versus tumor cells.

To determine whether BID translocates to the mitochondria in Jurkat cells coincubated with tumor cells, the cocultured cells were stained (Fig. 3) with anti-CD3-conjugated to Cy5 (magenta) to detect T cells, with Hoechst (blue) to assess nuclear morphology, with BID-specific Ab visualized by FITC-tagged secondary Ab, and with the MitoTracker dye CMXRos (red/orange). Using this image analysis procedure, multiple layers are viewed simultaneously, and only when three-dimensional colocalization occurs is a color shift detected. In control nonapoptotic Jurkat cells, BID was detected in the cytoplasm and in some nucleoli (Fig. 3A, middle panel). CMXRos-stained mitochondria in control cells were also detected in the cytoplasm, but with no colocalization with BID (Fig. 3A, right panel, red/orange). In Jurkat cells coincubated with tumor cells, BID was detected in the cytoplasm, in colocalization with the mitochondria, as assessed by the color shift to yellow (Fig. 3B). CD3 staining (magenta) served to distinguish lymphocytes from surrounding tumor cells. Similar translocation was observed in Jurkat cells treated with staurosporin (Fig. 3C) or agonistic anti-Fas Ab (Fig. 3D), which served as positive controls. Thus, control mitochondria stained with CMXRos (red/orange; Fig. 3A, right panel) shifted to a bright yellow color after translocation of and colocalization with BID-FITC (Fig. 3C, B-D, right panels). These results demonstrate that BID translocation to the mitochondria occurs under a variety of apoptotic stimuli, including that initiated by tumor cells.

A Role for Cytochrome c in Tumor-induced T-cell Apoptosis. To directly investigate the role of mitochondria in tumor-induced apoptosis of lymphocytes, we examined release of cytochrome c to the cytosol in Jurkat cells after coculture with tumor cells for 4 h. As shown in Fig. 4, release of cytochrome c to the cytosol (S100 fraction) was detected in Jurkat cells stimulated with anti-Fas Ab (Fig. 4A) or coincubated with tumor cells (Fig. 4B). In control untreated cells, cytochrome c was detected exclusively in the HM fraction containing the mitochondria. The observed release of cytochrome c was apoptosis mediated because there was no release of the inner mitochondrial membrane enzyme, cytochrome c oxidase (Fig. 4, A and B). No release of cytochrome c was observed in the apoptosis-resistant Jurkat cell line coincubated with tumor cells, consistent with these cells being resistant to both the Fas death receptor pathway and a mitochondrial pathway (VP-16).5 Taken together, these results suggest that the mitochondria are involved in tumor-induced apoptosis of T cells, and the release of cytochrome c to the cytosol may initiate a mitochondrial pathway of apoptosis.

Activation of Caspase-9 in Tumor-induced Apoptosis of T Cells. In cells treated with chemotherapeutic agents, release of cytochrome c from the mitochondria has been shown to cause activation of caspase-9. Because coincubation of Jurkat cells with SCCHN cells induced release of cytochrome c to T-cell cytosol, we examined whether the released cytochrome c was sufficient to activate caspase-9. Activation of caspase-9 in Jurkat cells coincubated with tumor cells was demonstrated by detection of its active subunits by immunoblotting (Fig. 5). Cleaved products of caspase-9 were also detected in Jurkat cells triggered by either anti-Fas Ab, staurosporin, or VP-16.

Effects of Inhibitors of the Apoptotic Mitochondrial Pathway on Tumor-induced T-cell Death. To elucidate the significance of the mitochondria in tumor-induced T-cell death, we investigated the effects of various inhibitors of mitochondrial pathways. ROS or oxidants are formed in the mitochondria but become toxic when present in excessive amounts, causing oxidative damage (24). To assess the significance of a redox imbalance, we used the antioxidants DPI (25 μM), a specific inhibitor of flavin-dependent oxidoreductase (25), and NAC (10 mM), a thiol antioxidant (26). Before incubation with tumor cells or agonistic anti-Fas Ab, Jurkat cells were treated with these antioxidants for 2 h. The effects of these inhibitors on tumor-induced apoptosis of lymphocytes were assessed by the JAM assay. As shown in Fig. 6A, loss in [3H]-labeled DNA in Jurkat cells coincubated with tumor cells was significantly reduced in the presence of either one of these antioxidants. However, apoptosis induced by agonistic anti-Fas Ab was significantly less affected. These results suggest that tumor-induced apoptosis of T cells involves the generation of ROS and is significantly inhibited by specific antioxidants.

Next, the effects of two mitochondria-specific inhibitors, BA and CsA, were examined. BA, a specific inhibitor of PT and a ligand of ANT in the inner mitochondrial membrane, can inhibit the apoptotic Δψm disruption (27, 28). CsA prevents mitochondrial PT by

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5 B. R. Gastman and H. Rabinowich, unpublished data.
blocking translocation of mitochondria matrix-specific cyclophilin D to the mitochondria inner membrane, thereby decreasing the sensitivity of mitochondrial megachannels to calcium ions (29). As shown in Fig. 6B, these inhibitors significantly (P < 0.01, Mann-Whitney U test) blocked mitochondria-dependent apoptosis of Jurkat cells induced by etoposide or diamide but had no effect on death induced by agonistic anti-Fas Ab tested at various concentrations. However, a significant inhibitory effect of either BA or CsA was detected on the level of tumor-induced apoptosis. These results further indicate that tumor-induced apoptosis of T cells has a component that is mitochondria dependent, in contrast to the apoptotic cascade induced by direct ligation of surface Fas.

To examine the significance of caspase-9 activation in the stimulated Jurkat cells, the cell-permeable peptide inhibitor of caspase-9, Ac-LEHD.CH0, was used. This reversible inhibitor had no effect on apoptosis induced by anti-Fas Ab but significantly inhibited the apoptotic effects of VP-16 or tumor cells (Fig. 6C). Because caspase-9-dependent VP-16-induced cell death was significantly blocked by Ac-LEHD.CH0, this inhibitor appears to mainly target the mitochondrial pathway of apoptosis. Thus, the partial inhibition of tumor-induced apoptosis of Jurkat cells by this peptide also suggests that the apoptotic process observed is partly mitochondria dependent.

Discussion

In the current study, we demonstrate that mitochondria are involved in apoptotic cascades induced in T cells by either Fas ligation or SCCHN. However, these two cascades are differentially susceptible to a panel of inhibitors of mitochondrial apoptotic events. Whereas Fas-mediated apoptosis in Jurkat cells is executed in the presence of mitochondria-specific inhibitors, tumor-induced apoptosis is partially...
inhibited, suggesting that it is significantly amplified by a mitochondria
dependent event was also induced by cross-linking of Fas, this or-
dependent because it is not inhibited by the pan-caspase inhibitor Z-
VAD.FMK (12, 13, 32). Potential mechanisms for the release of cytochrome c include opening of mitochondrial PT pores, the presence of specific channels for cytochrome c release, or mitochondrial swelling and rupture of the outer membrane, but without loss of mitochondrial membrane potential (33). Because mitochondria-dependent events were also induced by cross-linking of Fas, this organelle has been proposed to act as an amplifier of death receptor signaling (30, 34). Recent studies have highlighted the role of BID, a BH3 domain-containing proapoptotic Bcl-2 family member, in cytochrome c release (16, 20). On ligation of death receptors and caspase-8 activation, BID is cleaved and translocates to the mitochondria, where it induces the release of cytochrome c.

We and others have recently reported that solid tumors induce the Fas apoptotic pathway in interacting T lymphocytes (3, 4, 18, 19, 35–37). This Fas-mediated cell death may be induced directly by Fas ligand expressed on tumor cells (3) or by activation-induced cell death mediated by up-regulation of receptors and/or ligands of the Fas- or tumor necrosis factor-related apoptosis-inducing ligand pathways in T lymphocytes (38, 39). To date, tumor-induced apoptosis of T cells has only been implicated with death receptor pathways of apoptosis (40–42). In the current study, apoptosis-associated alterations in mitochondria served to confirm the involvement of mitochondria in the signaling phase of tumor-induced apoptosis, and a variety of inhibitors specific for different mitochondrial effector molecules served to reaffirm the significance of this amplification loop. The inhibitors included in this analysis targeted the generation of ROS, changes in mitochondrial transmembrane potential, or activity of caspase-9.

Mitochondria are the major source of oxidants, which are generated as a result of a decrease in coupling efficiency during electron chain transport (24). Generation of ROS is increased during apoptosis induced by a myriad of stimuli (43), suggesting that intracellular oxidation may be a general feature of the mitochondrial effector phase of apoptosis. Our results, which demonstrate an effective inhibition of tumor-induced apoptosis by antioxidants, suggest that in contrast to anti-Fas-mediated apoptosis, mitochondria are actively involved in tumor-induced T-cell death.

During the effector phase of mitochondria-dependent apoptosis, the inner transmembrane potential of the mitochondria collapses (44), indicating the opening of large conductance channels known as mitochondrial PT pores. The structure and composition of the PT pore, which is only partially defined, includes both inner membrane proteins, such as ANT, and outer membrane proteins, such as the voltage-dependent anion channel. The inner and outer membrane proteins operate in concert to create the conductance channels (34). Inhibitors
of the PT pore opening, including CsA, which binds cyclophilin D (associated with ANT), and BA, which also binds ANT, block the PT pore formation. Because these pharmacological inhibitors of the PT pore did not inhibit apoptosis induced by agonistic anti-Fas Ab but did inhibit the mitochondrial cascade initiated by VP-16, diamide, or tumor cells, the phase of PT pore formation appears to be central in the affected apoptotic pathways.

Caspase-9 knockout mice are resistant to apoptotic signals that stimulate the mitochondrial pathway (45), suggesting that caspase-9 plays a central role in mitochondria-dependent pathways of apoptosis. The presence of cleaved products of caspase-9 in tumor-induced apoptotic T cells and the inhibition of death by a caspase-9-specific inhibitor further demonstrate that a mitochondrial cascade plays a significant role in tumor-induced apoptosis of the Jurkat T cells.

BID cleavage and translocation to the mitochondria suggest that the observed mitochondria-dependent events induced in T lymphocytes by tumor cells are, at least in part, related to activation of caspase-8 by death receptors. In such a case, cross-communication between caspase-8 and caspase-9 would be related to the same triggering event and would serve to increase the efficiency of death induced by interaction with tumor cells. It would therefore be expected that inhibition of the mitochondrial amplification loop of caspase activation would attenuate a cascade initiated by death receptors. Interestingly, the various inhibitors of the mitochondrial effector phase of apoptosis used had no effect on cell death induced by ligation of Fas on the surface of Jurkat cells. These observations suggest that the mitochondrial amplification of the Fas cascade in Jurkat cells is not significant. However, each of the inhibitors used significantly hindered Jurkat cell death induced by SCCHN cells. These findings suggest that besides the Fas-mediated pathway, additional mitochondria-dependent cascades are involved in apoptosis of tumor-associated lymphocytes. Alternatively, it is possible that the Fas signaling mediated at the tumor microenvironment is weaker than that delivered by direct cross-linking of Fas on Jurkat cells by agonistic anti-Fas Ab. In the case of insufficient signal, a tumor-initiated caspase-8 cascade would be dependent on cleavage of BID and subsequent mitochondrial amplification of the apoptotic cascade. In any event, tumor-induced apoptosis of Jurkat cells appears to be significantly attenuated by inhibitors that specifically target mitochondrial effector molecules.

Scaffidi et al. (15) characterized the Jurkat cells used in their studies as type II, i.e., dependent on mitochondria for execution of Fas signaling, because in those cells the formation of the death–inducing signaling complex was not efficient. Although derived from the same source (46), uncloned Jurkat cell lines propagated for years by different groups are composed of variable mixtures of T-cell populations. Indeed, lines and clones of Jurkat cells resistant to Fas, tumor necrosis factor-related apoptosis-inducing ligand, or other apoptotic stimuli have been selected from apoptosis-sensitive Jurkat cell lines (18, 47, 48). In contrast to the Jurkat cell line used by Scaffidi et al. (15), the Jurkat cell line used in the current study was mitochondria independent for execution of apoptosis stimulated by the agonistic anti-Fas Ab. Similar characterization of Jurkat cells as mitochondria independent for Fas signaling has also been reported by others (49). Our findings of differential requirements for mitochondria in the execution of apoptosis of the same cell type suggests that the effector role of mitochondria is stimulus dependent.

In summary, the present study demonstrates that a mitochondrial cascade is contributing to the apoptotic mechanism induced in T cells by SCCHN. Blocking of this apoptotic loop may be important for the success of T-cell-based immunotherapeutic regimens in cancer.

References

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