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Attack the Tumor Counterattack-C-Flip Expression in Jurkat-T-Cells Protects Against Apoptosis Induced by Coculture with SW620 Colorectal Adenocarcinoma Cells

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Background. **Cancer development relies on a variety of mechanisms that facilitate tumor growth despite the presence of a functioning immune system, employing different mechanisms to escape immune rejection. Tumors may eliminate tumor-infiltrating lymphocytes and suppress anti-tumor immune responses, a process called "tumor counterattack," based on activation-induced cell death *via* the FAS/FAS-ligand system. To overcome this tumor-cell survival strategy, we examined the hypothesis that the sensitivity of FAS mediated apoptosis of Jurkat-T-cells can be suppressed by FLIP transfection of Jurkat-T-cells.**

Materials and Methods. **Jurkat-T-cells were transfected with the FLICE-inhibitory protein FLIP in order to bestow them with a resistance to FAS-receptor-mediated apoptosis. FLIP-transfected and non-transfected Jurkat-T-cells were grown in coincubation with SW620 cells and the rates of apoptosis measured *via* FACS-analysis of Annexin-V.**

Results. **First, the tumor-counterattack described in the literature was confirmed. About 20% of Jurkat-T-Cells underwent apoptosis in coculture with SW620 cells. After coincubation of SW620 cells with FLIP transfected Jurkat-T-cells the apoptotic rate was significant reduced at levels below 4%.**

Conclusion. **Transfection of Jurkat-T-cells with FLIP reduces the sensitivity of Jurkat-T-cells to FAS-mediated apoptosis and may lead to an improved capability to antagonize the inherent tumor survival strategy of SW620 cells.**

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Key Words: **apoptosis; colon carcinoma; tumor counterattack; immunotherapy; gene therapy; FLICE-inhibitory-protein (FLIP).**

INTRODUCTION

The establishment and progression of malignant cells to the formation of a tumor is dependent on several mechanisms. An intact immune system is capable of detecting tumor cells and destroying them through cell-to-cell or cytotoxic mechanisms [1–5]. The so-called tumor-infiltrating lymphocytes (TIL) migrate to and then enter the tumor; these represent the most important, specific immune response against the tumor [3]. Various subpopulations of lymphocytes can be detected in tumor samples, including CD8+, CD4+, natural killer cells (NK), macrophages, and dendritic cells (DCs) [6–10].

During tumorigenesis, tumor cell populations first acquire properties enabling their local invasion into surrounding organ structures as well as their hematogenic and lymphatic metastasis [3, 11]. Possible explanations for how tumor cells can survive and multiply as a quantitative minority among differentiated cell populations in the lymphatic and circulatory systems represent so-called "escape strategies," which are developed by a wide variety of tumors during tumorigenesis [3, 12–15].

One of the recognized tumor escape strategies lies in altering of the expression of MHC molecules. Many known tumor types only express MHC Class I molecules and not Class II. This allows them to escape recognition by Th-2 helper cells (Th2) [6, 7]. Research into auto-immune diseases has described the FAS/FAS-ligand system, which

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plays a key role in the lymphocytic homeostasis of the immune response [16, 17]. To avoid an excessive immune response, activated lymphocytes increase the expression and sensitivity of their FAS receptors, binding to which begins the process of apoptosis in the cells. This autoregulatory suppression of the immune response is mediated by FAS-ligand-expressing CD8+ and Th1 cells [5]. It is exactly this mechanism of autoregulatory immune suppression that could represent a survival strategy for tumor cells.

Various tumor entities express FAS-ligand in high levels and are not themselves sensitive to FAS-mediated apoptosis [13–15, 18–22]. As such, tumor cells that express FAS ligand stimulate apoptosis in tumor-infiltrating lymphocytes, thereby removing one of the immune system's mechanisms for the destruction of tumor cells.

During the search for regulatory mechanisms of the FAS/FAS-ligand system, Thome *et al.* described a new family of proteins, FLIP, (FLICE-inhibitory protein [FLICE ≈ FADD-like interleukin-1ß-converting-enzyme]) [23]. vFLIP proteins are viral inhibitors and are found in various herpes viruses including Karposi's-sarcoma-associated human herpes virus 8 and the human molluscipox virus [23–25]. The protein $FLIP_S$ contains two death effector domains (DED), which can interact with the FAS-associated death domain (FADD) and caspase-8 and possibly caspase-10.

The binding of FAS-ligand on the FAS receptor leads to a new re-formation of these transmembrane and intracellular proteins to a death-inducing signaling complex (DISC) [26–28]. An overexpression of FLIP leads to the inclusion of FLIP in the DISC. This inclusion blocks the intracellular cascade, leading to activation-induced cell death (AICD) [5, 29–31], whereby the FLIP-mediated activation of caspase-8 inhibits the AICD [32] (Fig. 1).

Moreover, the higher the expression of FLIP and its integration into the DISC are, the higher the resistance of the cell to apoptosis. This may involve a competitive effect of caspase-8 and FLIP for integration into DISC. $FLIP_S$ and $FLIP_L$ are able to inhibit apoptosis *via* all known death receptors, FAS, TRAMP (wsl/DR-3/APO-3), TRAIL-R (DR-4), and tumor necrosis factor receptor 1 (TNF-R1) [32].

In summary, c-FLIP is an anti-apoptotic molecule for which a high level of expression quantitatively correlates to resistance to apoptosis [33].

The innovative approach of this study lies in inhibiting the FAS-ligand-expressing tumor cells' ability to eliminate FAS-sensitive T-cells, thereby disrupting the tumor's escape strategy. To that end, Jurkat-T-cells were transfected with FLIP and their rates of apoptosis in coincubation with SW620 colorectal adenocarcinoma cells examined.

MATERIALS AND METHODS

Cell Culture

Jurkat T-cells are consistent with acute T-cell leukaemia cells and appear morphologically identical to lymphoblasts; Jurkat T-cells (American Type Culture Collection (ATCC), Manassas, VA, order number: CRL-2063). SW620 colorectal adenocarcinoma cells were isolated from a lymph node metastasis of a 51-y-old Caucasian as was

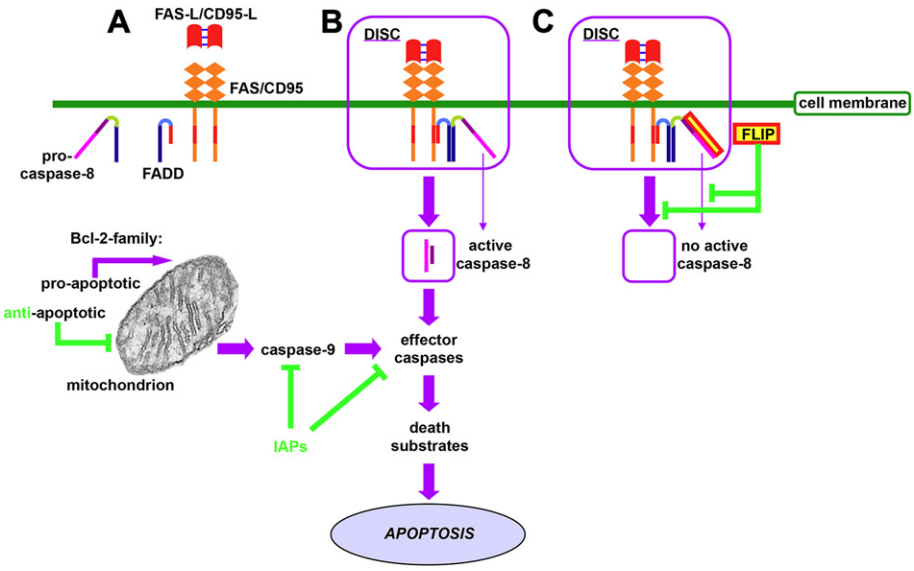

FIG. 1. Apoptosis signaling *via* the Fas/Fas-Ligand pathway. (A) Shows the different proteins FAS, FAS-Ligand, FADD, and pro-caspase-8 that are involved to create the death inducing signaling complex (DISC). (B) Shows the binding of FAS-ligand on the FAS receptor leading to DISC formation and thereby activation of pro-caspase-8 into active caspase 8, which is the key protein in the activation-induced cell death process (AICD). (C) Shows prevention of activation of caspase-8 by FLIP binding to the DISC. Apoptosis can also be inhibited on different intracellular levels by other anti-apoptotic proteins (shown in green). (Color version of figure is available online.)