

Staurosporine Modulates Radiosensitivity and Radiation-Induced Apoptosis in U937 Cells

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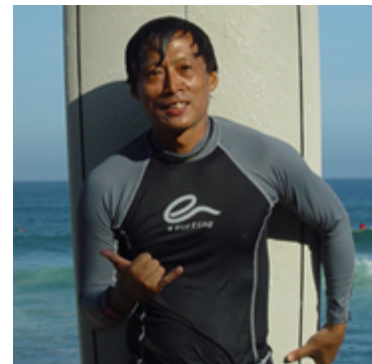
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Ionizing radiation (IR) is one of the most effective tools in the clinical treatment of cancer because cancer cells generally show higher sensitivity to radiation than do noncancerous tissues. G₂/M arrest and apoptosis are commonly observed in cells after DNA damaging treatment such as irradiation. On the other hand, DNA injuries can be restored by various repair systems during G₂/M arrest. Thus, when the duration of G₂/M arrest is not sufficient, cells with DNA injuries display immature mitosis or mutagenesis. Furthermore, damaged cells could be eliminated as apoptotic cells if the DNA injury is lethal. Although it is not fully understood how DNA damage causes cells to undergo apoptosis at the G₂/M checkpoint, this is one of the most important mechanisms in the radiation therapy of malignant tumors.



Apoptosis is a highly regulated process that controls normal development and homeostasis of multicellular organisms. Inability to control the tightly regulated apoptosis causes many human diseases such as cancer, autoimmune diseases, and various neurodegenerative disorders. Depending on the stimulus that initiates apoptosis, different caspase cascades, the core of the apoptotic program, are activated. Caspases are a family of specific cysteine proteases, and their activity is critical in the intracellular execution of programmed cell death, apoptosis. Many members such as caspase-1, caspase-2, caspase-3, caspase-8, and caspase-9 have been identified. Among them, caspase-3 plays a major role in the effector phase of apoptosis induced by a variety of stimuli.

Apoptosis induced by irradiation could be found in many cell types and is also a significant mechanism of tumor cells under radiotherapy. However, cellular resistance could occur through overexpression of antiapoptotic proteins or the loss of apoptosis-inducing proteins such as tumor suppressor protein p53. For instance, radioresistance, cellular resistance to irradiation, of tumor cells lacking p53 proteins may lead to diminish the ability to undergo apoptosis in vivo and in vitro. The status of p53 is pivotal for the response of tumor cells to IR. Irradiation of cells with wild-type p53 gene elevates the level of cellular p53 protein and regulates the expression of a variety of downstream effector genes. It has been reported that p53 mutation or p53-deficient cells could be observed in fifty percent of human cancer cells. Thus, the use of chemical modifiers as radio-sensitizers in combination with low-dose irradiation may increase the therapeutic effect by overcoming a high apoptotic threshold.

Abrogation of the G2 checkpoint has been associated with the sensitivity of tumor cells to DNA-damaging agents. Staurosporine (STP) was originally isolated from a *Streptomyces* species as an inhibitor of protein kinase C (PKC). STP and its analogues have anti-tumor properties alone and have also been shown to abrogate the G2 checkpoint and to sensitize tumor cells to DNA-damaging agents. However, the molecular basis for the effects of STP in combination with the irradiation of leukemia cells has seldom been thoroughly studied. The G2/M arrest means the existence of DNA damage repair prior to potential activation of apoptosis and may be a crucial determinant of radioresistance. Some studies implicated that chemical compounds capable of abrogating G2/M arrest and stimulating apoptosis are clinically available to override radioresistance. According to these studies, we combined the treatment of low-dose STP with low-dose irradiations in the current study to overcome the radioresistance and G2/M arrest induced by low-dose irradiation in p53 deleted cancer cells. Our results indicated that the combined treatment with 10 nM STP and 5 Gy IR for 18 hr could induce remarkable apoptosis when compared to STP or IR treatment alone (Fig.1). Meanwhile, G2/M arrest was overridden, and the G1 phase proportion was increased (Fig. 2). We believe that STP can override the G2/M arrest induced by low-dose (5 Gy) IR and thus lead to earlier apoptosis. Our data implicated that the balance between the extent of DNA damage and the duration of G2/M arrest might determine whether irradiated cells would survive or undergo apoptosis.

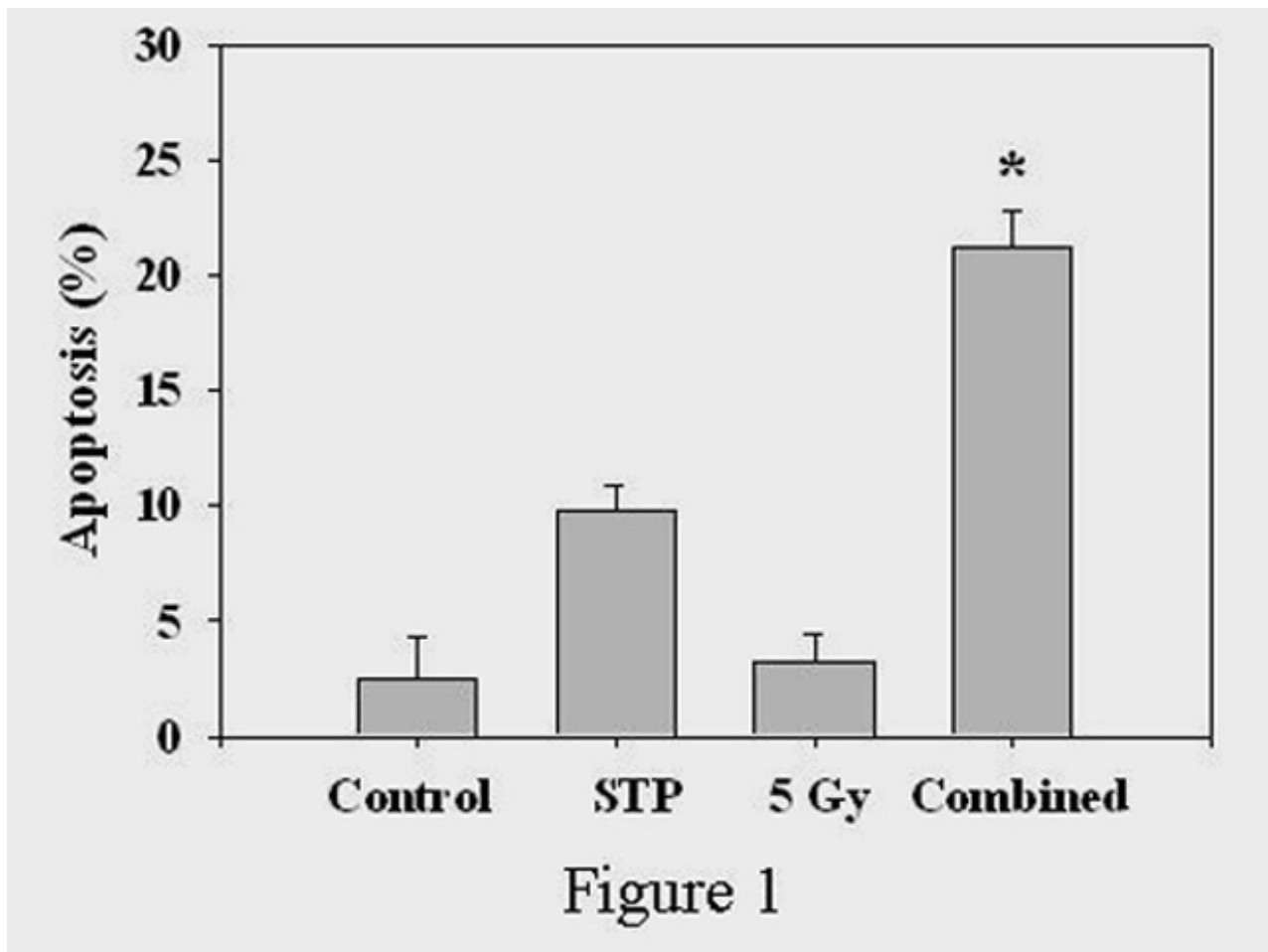


Figure 1. Quantification of apoptotic cells treated with 10 nM STP and 5 Gy IR alone or combination for 18 hours. Apoptotic fraction was recognized as sub-G1 population of cell cycle measured by flow cytometry.

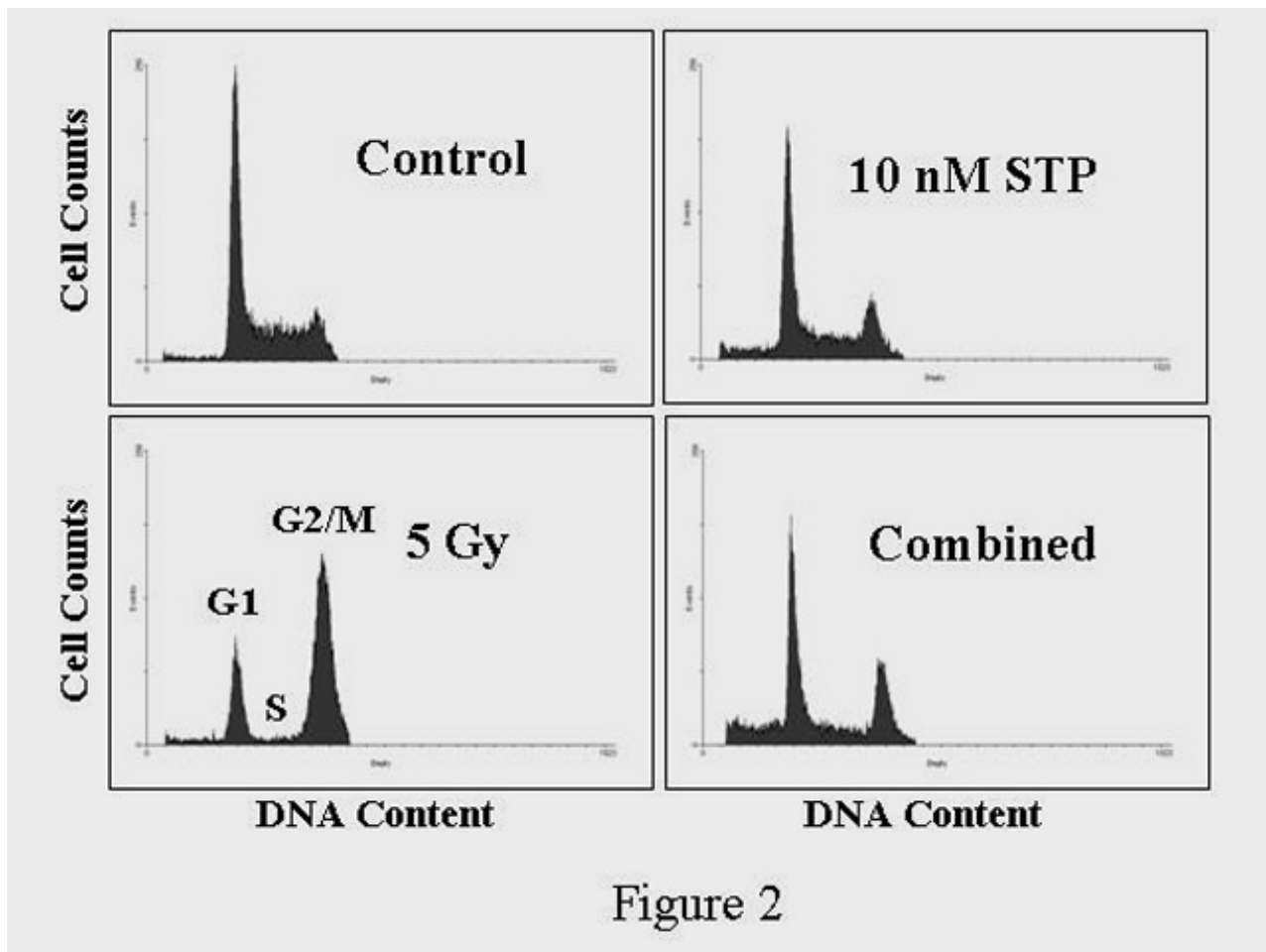


Figure 2

Figure 2. Cell cycle progression in U937 cells treated with STP and IR. Proportion of cell cycle phase was analyzed by flow cytometry. U937 cells were treated with STP, IR or combination for 18hrs.

The combination of different antitumoral treatment modalities is advantage to limit unspecific toxicity often observed by an exceeding high single treatment regimen. Our results indicated that combined treatment led to an increased apoptotic cell death in U937 cells, which is correlated with the phosphorylation of JNK, the activation of caspases, the increase in Bax and decrease in Bcl-XL levels, and the loss of mitochondria membrane potential and the release of cytochrome c. In addition, we also observed a marked induction of p21 and an inhibition of cyclin A in cells with combined treatment. Meanwhile, the G1 phase was increased. According to the results of this study, radiation therapy should be an effective strategy against cancer cells in combination with stourosporine via sensitizing cells to radiation-induced apoptosis. It is encouraging that chemicals such as STP that abrogate G2/M arrest and stimulate apoptosis after DNA damaging treatment could be clinically available. Moreover, the combination of STP and radiation should be further investigated as potential strategy for enhancing the response of p53-deficient, radioresistant tumors cells.