Sulfasalazine Inhibits IL-2 Expression in Ovarian Cancer Cells

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Abstract: Ovarian cancer has become threatening signal for women in the present world. Lack of specific biomarkers, early occurrence but late diagnosis has made the screening difficult. Hence there is a need to shift the study towards ovarian cancer therapy. Wide ranges of anticancer drugs are available with specific targets but have many side effects. The IL-2 an inflammatory cytokine and is highly expressed in cancer cells. Sulfasalazine an anti-inflammatory drug inhibits NFkB. In this study we investigated the effect of sulfasalazine on IL-2 expression in ovarian cancer cell line SKOV-3 and the underlying mechanism. We observed sulfasalazine as a potential candidate to treat ovarian cancer by diminishing the levels of IL-2.

Key words: Sulfasalazine, IL-2 and ovarian cancer.

INTRODUCTION
Ovarian cancer is the most lethal among gynecologic malignancies with high rate of occurrence every year. It is the cause of cancer death in women. It is often diagnosed at later stages hence difficult to cure. As there are less biomarkers available which makes screening difficult current research has focused on treatment strategies rather than the preventive measures. Treatment of ovarian cancer has become challenging in the present decade. Surgery, radiation and chemotherapy are widely used treatment strategies(1). For an effective therapy understanding the mechanism of cancer initiation, progression and the genes, signaling pathways involved is highly essential. AKT1, BRCA2, BRCA1, CDH1 and BARD1 are few genes known to be associated with ovarian cancer. Dysregulation of various oncogenes and tumor suppressor genes is responsible for ovarian cancer. Angiogenin, angiopoietin-2, ICAM-1, IL-8, IL-6, IL-10, leptin, IL-6R, MIF NAP-2, MCP-1 and osteoprotegerin (OPG) were elevated in most malignant ovarian ascites (2). Inflammatory response is the main cause of cancer mediated by different cytokines and interleukins. Wide varieties of drugs like cisplatin, taxane, paclitaxel and docetaxel are available for the treatment of ovarian cancer. These drugs target different genes associated with ovarian cancer (3).

Sulfasalazine (SUFZ) is one such potent anti-inflammatory drug used for the treatment of inflammatory bowel disease, rheumatoid arthritis, ulcerative colitis and crohn’s disease. It has good safety record in pregnancy hence is widely used synthetic drug with combined activities of sulfapyridine antibiotic and 5-aminosalicylic acid (5-ASA). Its anti inflammatory action comes from its ability to inhibit nuclear factor-κB (NF-κB) which is essential to induce pro-inflammatory cytokines IL-8, IL-1β, TNF-α and IL-6 (4).

Sulfasalazine inhibited NF-κB activity in SW620 colonic epithelial cells very effectively when compared to its individual moieties sulfapyridine antibiotic and 5-aminosalicylic acid (5-ASA)(5). Sulfasalazine inhibits NFkB and thus mediates immununospression by inhibiting the production and secretion of different cytokines. IL-2 is the most potent cytokine reported to be highly expressed in cancer cells. The primary role of IL-2 is to activates natural killer cells, B- and T-lymphocytes and aids in their growth and differentiation. It helps in self tolerance and maintains immune hemostasis.. In tumor microenvironment IL-2 activates the immune system and evades tumor cells. IL-2 is reported to be effective against metastatic melanoma and renal cell carcinoma (6). IL-2 enhances cytolytic functions of natural killer cells. IL-2 initiates signaling pathway by binding to its receptor IL-2R. IL-2 protein, interleukin-2 (IL-2) receptors, and mRNA for IL-2 are reported to be highly expressed in human carcinomas (7). Immunohistological analysis of IL-2 expression in squamous cell carcinoma of the head and neck showed positive correlation of il-2 expression with tumor grade. Tumor cells showed expression of IL-2 within the golgi complex. The IL-2 expression is intense in actively dividing cells and is associated with cell proliferation. Cervical cancer cells are observed to express IL-2R and also produce IL-2 which aids in their survival and proliferation. Hence it acts as an autocrine growth factor and possibly contributes to
immunological escape (8). The targeted delivery and sustained release of IL-2 at the site of tumor was achieved with the use of nanolipogels (9). Combined therapy with specific inhibitors against other receptors like TGF-β diminished tumor growth. IL-2 also mediates death signals and induce apoptosis in autoreactive T cells. IL-2 and IL-2 receptor-deficient mice exhibit lethal autoimmunity. IL-2 controls autoimmunity through the production of CD4+CD25+ T regulatory (Treg) cells (10).

In the current study, we investigated the role of sulfasalazine in regulating IL-2 expression in the human ovarian cancer cell line SKOV-3. We found that sulfasalazine inhibited IL-2 expression in dose dependent manner. In SUFZ treated cells the IL-2 levels were decreased. SUFZ induced cell death in ovarian cancer cells by inhibiting IL-2 expression. Our study revealed a novel mechanism by which sulfasalazine inhibits ovarian cancer cell growth and provides a new clue for the ovarian cancer therapy.

MATERIALS AND METHODS

Materials

Sulfasalazine obtained from (TCI chemicals Ltd, India). Fetal Bovine Serum (FBS) PBS, penicillin, Dulbecco’s Modified Eagle Medium (DMEM) and Trypsin-EDTA were procured from Himedia Laboratory (P) Ltd, India. IL-2 antibody from Cistron biolabs, India. Secondary mouse and rabbit antibodies conjugated with HRP from Santa Cruz (TX, USA).

Methods

Cell lines and cell culture

SKOV-3 human ovarian carcinoma cell line was obtained from the National Centre for Cell Sciences (NCCS), Pune, India. High glucose (4.5 g/l) DMEM medium with 10 % fetal bovine serum, 100 mg/ml Streptomycin, 100 units/ml Penicillin was used for cell culture and the cells were maintained at 37 °C under 5 % CO₂ and 95 % air. Cells were trypsinized and subcultured every alternate day.

Sulfasalazine (SUFZ) treatment:

10mM stock solution of SUFZ was prepared and further dilutions were made in cell culture media. Cells were treated with 20µM and 50µM of SUFZ for 6hours.

ELISA (Enzyme-linked immunosorbent assay):

SKOV-3 cells both treated and untreated were lysed and the whole cell fraction was collected after SUFZ treatment. Equal amount of lysates were added to plate using coating buffer. Now the wells were washed with wash buffer after 2 hours of incubation. The wells were blocked and incubated with primary antibody for two hours at room temperature. After 3 washes analytes were incubated with secondary antibody for 1hour. 200 µL/well TMB substrate (Abcam) was added and stop solution of 50ul/well was used to terminate the reaction and the relative intensity of expression was quantified from absorbance readings at 450nm.

Statistical analysis

GraphPad PRISM software and one-way ANOVA was used for statistical analysis of data. Statistical significance was evaluated by calculating P-values. Differences where P<0.05 were considered statistically significant. (*P<0.05, **P<0.01, ***P<0.001).

RESULTS

Sulfasalazine (Figure 1) is an anti inflammatory drug under the trade name Azulfidine. 5-aminosalicylic acid (5-ASA) and sulfapyridine are the two moieties of sulfasalazine. It inhibits cysteine transport into the cancer cells. Inadequate amounts of cysteine leads to the deficiency of antioxidants like glutathione.

Figure 1. Chemical structure of Sulfasalazine.

Effect of sulfasalazine (SUFZ) on IL-2 production in SKOV-3 celline:

IL-2 is an inflammatory cytokine that gets induced upon microbial or unnatural threat and activates T-lymphocytes. The role of IL-2 is studied in different cancers like prostate, melanoma liver and neuroblastoma. In human cancer cells the expression of cytoplasmic IL-2 varied with the cell cycle phase. Another study showed the use of antisense cDNA to IL-2R inhibited the growth of tumor cells (11). As sulfasalazine, an anti-inflammatory drug that inhibits NFkB and is essential for various cytokines production like IL-2
we determined to check the effect of SUFZ on IL-2 expression using ELISA in SKOV-3 cell line.

Figure 2: IL-2 expression in SKOV-3 cell line upon sulfasalazine treatment

We observed a decrease in IL-2 levels with increasing concentration of sulfasalazine. Hence SUFZ inhibits cancer cell growth by targeting IL-2 production which is reported to express at high levels in cancers.

To further identify the possible mechanism and targets of IL-2, we analyzed the protein network of IL-2 using STRING database to study the mechanism of regulation. We observed FOS and IFNG, well-reported proto oncogenes to mediate IL-2 signaling (Figure 3).

Figure 3 Protein network of IL-2 detected from STRING

Hence IL-2 is known to interact with various partners that have role in immune response, cell proliferation and tumor progression.

DISCUSSION & CONCLUSION

The development of cancer is a multi-step process with multiple factors involved in it. Altered expression or function of tumor suppressor genes and oncogenes is the main cause of cancer progression. Inflammation also has an emerging role in cancer development. Most of the tumors are initiated from infection or wild inflammatory response. The inflammatory cells release various cytokines that promote angiogenesis, survival, invasion and helps in remodeling ECM. IL-2 is one such gene that activates immune response and promotes tumorigenesis. In synchronized cells, there is an inverse relation between expression of IL-2 and p27. There is a significant increase in p27 as well as p21 expression when the endogenous synthesis of IL-2 was blocked using the IL-2 specific antisense oligonucleotide in tumor cells. IL-2 is essential in regulating the expression of p27 and p21 and thus controls cell cycle progression of tumor cells. Upon DNA damage or antimitogenic signals p21 and p27 induce cell cycle arrest by binding to cyclin-CDK complexes, but IL-2 highly expressed in cancer cells regulates the p21 and p27 expression and bypasses the cancer cells from cell cycle arrest and cell death. Hence in tumor cells, endogenous IL-2 might mediate the cell cycle progression by regulating the expression of CDK inhibitors. IL-2 also interacts with other oncogenes like FOS and IFNG and thus regulates various signaling pathways that leads to cancer progression.

This current study focuses on the immune modulatory effects of sulfasalazine in ovarian cancer cell line SKOV-3. Our results showed diminished expression of IL-2 on treatment with sulfasalazine. SUFZ induce apoptosis in ovarian cancer cells by decreasing the expression of IL-2. IL-2 inhibits NFKB which is essential to activate pro apoptotic genes and also regulates tumor suppressor genes. Hence SUFZ might induce apoptosis by activating proapoptotic genes and suppress antiapoptotic factors via IL-2 – NFKB signaling pathway. Further studies are required to confirm our hypothesis. Thus sulfasalazine can be a new ray of hope for ovarian cancer treatment. As most of the present drugs cause several side effects, combinational therapy is used to reduce side effects and to increase specificity. Our study also gives an advantage to choose appropriate drug for combinational therapy.

In summary, we disclosed a new mechanism underlying the effect of SUFZ on ovarian cancer.

Conflict of interests: The authors declare that there are no conflicts of interests.
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