**Flavokawain B Induces Apoptosis in Synovial Sarcoma Cell Lines**

1Sakai, T; 2Eskander, R; 2Guo, Y; 1Kim KJ; 1Mefford J; 1Hopkins J; 1Bhatia NN; 2Zi, X; +Hoang BH
+University of California, Irvine, Orange, CA,

bhhoang@uci.edu

**INTRODUCTION:**

Synovial sarcoma (SS) is a soft tissue sarcoma which occurs frequently in adolescents and young adults. It comprises 5-10% of all soft tissue sarcomas. Histologically, this tumor is divided into two predominant subtypes: monophasic - entirely composed of spindle cells and biphasic - composed of both spindle cells and epithelial cells. The outcome of treatment for SS is poor due to its resistance to radiation and chemotherapy, with 5-year survival rate ranges from 55% to 76%. Although surgical resection and adjuvant radiotherapy are the mainstays of SS treatment, SS have high rates of local recurrence and systemic metastasis. The 5-year local-recurrence and distant-recurrence were 12%, 39%, respectively. Furthermore, no therapeutic options have proven effective for advanced stage or unresectable SS. However, this may represent an unique opportunity for chemoprevention approach to reduce the recurrence of SS.

Kava (Piper methysticum) is an ancient crop of the western Pacific. The root extract of kava has been a part of the Pacific Islanders’ culture for thousands of years, serving as a beverage, medicinal purposes and in socio-religious functions similar to wine in Western cultures. Consumption of traditional aqueous kava preparations correlates with low and uncommon sex ratios of cancer incidences in three kava-drinking countries: Fiji, Vanuatu, and Western Samoa. Recently, flavokawain B (FKB), a kava chalcone, was demonstrated to cause apoptosis in prostate cancer cells, and also to inhibit growth of human squamous carcinoma cells. 2

The current study, we examined the effects of FKB on two SS cell lines and hypothesized that this compound has a potential as a chemopreventive agent for SS by enhancing apoptotic mechanism.

**METHODS:**

Synovial sarcoma cell lines SYO-I and HS-SY-II, normalized according to cell number, were treated with different concentration of FKB. Cell viability was assessed by MTT assays. Anchorage-independent growth was examined by soft agar assay. The data are shown as mean number of colonies ± SEM at 14 d for SYO-I, 28 d for HS-SY-II after the start of cell seeding. Intrinsinc (mitochondrial) and extrinsic apoptotic pathways were examined using the Caspase 3/7,8 and 9 assays. Western blot analysis and real time RT-PCR for apoptosis-related death receptor 5 (DR5), Survivin, Bim and Puma were performed. The data are presented as means ± standard errors (SE). The level of significance was set at a p value < 0.05. Comparisons of differences between treated and control groups were performed using student’s t test. All statistical tests were 2 sided.

**RESULTS SECTION:**

MTT assays showed that FKB, at a concentration of 5.0 μg/ml, inhibits the growth of both SYO-I and HS-SY-II cell lines by 69% and 77%, respectively (Student t-test, p<0.05).

In soft agar, both SYO-I and HS-SY-II cell lines treated with FKB formed significantly less colonies than those treated with 0.1% DMSO as a vehicle control (p<0.01) in a dose-dependent manner.

FKB increases caspase 8, 9 and 3/7 compared to controls in both cell lines, suggesting that both death receptor- and mitochondrial-mediated apoptotic pathways are activated.

FKB treatment of both cell lines resulted in increased mRNA expression of DR5 and the mitochondrial pro-apoptotic Bim and Puma, while down-regulating an inhibitor of apoptosis protein (IAP), survivin. Analogously, FKB treatment resulted in a significant increase in protein expression for DR5 and downregulation of survivin expression.

Taken together, these results strongly imply that FKB activates both extrinsic and intrinsic pathways, inhibiting a robust apoptotic mechanism against synovial sarcoma.

**DISCUSSION:**

In this study, we demonstrated that flavokawain B (FKB) induced apoptosis in two synovial sarcoma (SS) cell lines (SYO-I and HS-SY-II). Previously, we reported that FKB induced apoptosis in androgen receptor-negative, hormone-refractory, prostate cancer cells (HRPC). 1

Recently, FKB was also reported to cause apoptosis in human squamous carcinoma cell lines. 2 However, to the best our knowledge, there have been no reports on the efficacy of FKB on sarcoma cell lines, including synovial sarcoma (SS). Our results demonstrated that FKB promotes apoptosis by targeting both pro and anti-apoptotic proteins in a dose-dependent manner. The apoptotic mechanisms of FKB in SS are similar to those in the previous report using HRPC. 1 FKB induced apoptosis via activation of caspase-3/7, 8 and 9 in both SYO-I and HS-SY-II cell lines. FKB induced apoptosis with an increased expression of proapoptotic markers: death receptor-5 (DR5), Bim and Puma, and a decreased expression of inhibitors of apoptosis: survivin. Increased DR5 expression by FKB activates the death receptor mediated apoptotic pathway. For example, a DR5 ligand, known as the Tumor Necrosis Factor Related Apoptosis Inducing Ligand (TRAIL) is considered an effective anticancer agent. Although it selectively induces apoptosis in a variety of tumor cells, it is relatively nontoxic to normal cells. The mechanism for FKB mediated DR5 expression remains unclear.

In addition, FKB was found to increase the expression of Puma and Bim in both SYO-I and HS-SY-II cell lines. DR5 and Puma are common p53 target genes. Interestingly, in comparison to other tumor types, synovial sarcomas display a remarkably low number of mutations in the p53 gene, implying that defects in upstream pathways may be responsible for loss of p53-mediated tumor suppression. We found that FKB did not change the expression of p53 in SS (data not shown). Therefore, it is less likely that FKB activates p53 for its effect on DR5 and Puma expression. Bim directly initiates BAX-mediated mitochondrial apoptosis via binding to and stabilizing the α-helix of the BCL-2 domains of BAX.

FKB decreased the expression of an inhibitor of apoptosis protein, survivin, in both cell lines in a dose-dependent manner. Survivin is well-known as one of the most tumor-specific genes in the human genome. Despite discussion and investigation into the apoptotic inhibitory effects of survivin, the precise mechanism of apoptosis inhibition is still a matter of discussion.

Despite surgical resection with/without adjuvant radiotherapy and/or docorubicin-based chemotherapy, the long term prognosis for patients diagnosed with synovial sarcoma is poor. Our data suggest that FKB may have a potential usefulness as a less toxic chemopreventive agent for reducing the recurrence of SS. To realize this possibility, further preclinical animal studies examining the in vivo anti-tumor efficacy of FKB against SS, as well as the potential side effects of this agent are required. In addition, FKB may have synergistic inhibitory effects with chemotherapeutic drugs on the growth of SS cell lines, which will be further explored in future.

**SIGNIFICANCE:**

These results illustrated that FKB exhibited robust mechanism in induction of apoptosis in SS. FKB may represent a new chemopreventive strategy for patients with SS and deserves further investigation as a potential adjuvant treatment of this malignancy.

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**REFERENCES:**