S.VI.1.

Molecular evidence to the preventive and therapeutic potentials of Ashwagandha leaves

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Ashwagandha (*Withania somnifera*) is an important and popular Ayurvedic herb used in Indian traditional home medicine. It has been known to possess various therapeutic and health-promoting potentials of which the molecular mechanism(s) remain obscure. We screened Ashwagandha leaf extracts for anticancer activity in *in vitro* and *in vivo* assays, and have found that the alcoholic (i-Extract) and water (WEX) extracts of Ashwagandha leaves possess considerable anticancer activity. Bioactives for these activities were identified as Withanolides, Withanone (Wi-N) and Withaferin A (Wi-A) in the i-Extract, and Triethylene Glycol (TEG) in the WEX. In cell culture models, these components caused cytotoxicity to a variety of human cancer cells. Molecular insights into their mechanism of action revealed that they cause (i) activation of tumor suppressor genes and (ii) induction of oxidative stress. Based on our studies, we formulated a combination of Wi-N and Wi-A with potent anti-metastasis activity and have also developed a method of extraction that yield all the three bioactive components from the leaf powder, and hence is proposed to serve as a cheap, economic anticancer drug especially where modern medicine is either not available or is limited by severe side effects. Furthermore, low doses of the extracts and some of the constituents were seen to possess anti-stress, anti-aging and neuro-protective activities. Molecular mechanism of these activities will be discussed.

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Key words: Ashwagandha; anticancer; neuroregeneration; anti-aging; anti-stress; active components; molecular mechanism

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S.VI.2.

Mitochondria – a promising target in colon cancer prevention

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Recent reports show the involvement of mitochondrial fission protein dynamin-related protein 1 (DRP1) in progression of cancer including colorectal cancer (CRC). We observed that butyric acid (BA) causes DRP1-mediated G2/M phase arrest and apoptosis in human CRC cells. BA is the byproduct of anaerobic microbial fermentation inside the gastrointestinal tract that could reach up to 20 mM, which can inhibit the growth of various cancers. The cell cycle arrest was associated with mitochondria-mediated apoptosis accompanied by a decrease in survivin and Bcl-2 expression, and generation of reactive oxygen species (ROS). DRP1 down-regulation was noticed in mitochondria which later became drastically reduced in both mitochondria as well as cytosol. Cyclin B1-CDK1 complex activates DRP-1 by its phosphorylation at ser616; both were strongly reduced by BA treatment. DRP1 was found to be regulated by apoptosis. DRP1 overexpression increased cell proliferation rate which was reversed by BA treatment involving cell cycle arrest. DRP1 overexpression in CRC cells resulted in pro-survival signaling which was inhibited by BA treatment. DRP1 increased cell migration and invasion, and this ability was strongly reduced by BA involving modulation of Snail and Slug leading to increased expression of E-cadherin and reduced expression of vimentin. DRP1 increased the colonosphere formation frequency indicating the emergence of tumor-initiating cells or cancer stem-like cells which was suppressed by BA. These results suggested that DRP1-mediated mitochondrial fission and EMT could be novel targets in CRC prevention by BA, a product of anaerobic fermentation of dietary fibers in gut.

Key words: colon cancer; mitochondrial fusion/fission; DRP-1, butyrate; EMT

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S.VI.3.

Dietary polyphenols target protein kinases for cancer chemoprevention

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Protein kinases regulate a variety of cellular processes by phosphorylating multiple target proteins. Although the activities of protein kinases are stringently regulated in homeostasis system, they are deregulated under pathological conditions, leading to perturbation of protein kinase-mediated cell signaling pathways and resulting in various disorders including cancer and inflammation. The data from Human Genome Project has revealed that more than 500 protein kinases are encoded in the human genome, thereby creating an enormous repertoire of potential targets for cancer chemoprevention. Interestingly, accumulated data have suggested that dietary polyphenols, rich in vegetables and fruits, play an important role in cancer chemoprevention with multiple mechanisms. Recent data from our and other groups have revealed that dietary polyphenols could directly interact with some protein kinases central to cellular signaling in carcinogenesis. These polyphenolic compounds could inhibit carcinogen-induced phosphorylation of some protein kinases such as MEK, AKT and JAK with different affinity spectrum and selectivity in binding sites of protein kinases, suggesting that dietary polyphenols possible target the carcinogenic protein kinases to exert the chemopreventive effects. In this conference, I will represent our typical data on the interactions of polyphenols-protein kinases, and discuss the impact of dietary polyphenols targeting protein kinases on cancer chemoprevention.

Key words: dietary polyphenol; protein kinase; cellular signaling; cancer chemoprevention

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S.VI.4.

Zapotin (5,6,2′,6′-tetramethoxyflavone) modulates the crosstalk between autophagy and apoptosis pathways in cancer cells with overexpressed constitutively active PKCε

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PKC epsilon, a member of the protein kinase C family, revealed high transforming potential and the ability to increase cell migration, invasion and metastasis. The key role in maintaining the cellular and tissue homeostasis is carried out by programmed cell death (PCD). Autophagy and apoptosis are two important catabolic processes regulating the balance between cell death and survival.

Zapotin (5,6,2',6'-tetramethoxyflavone) is a natural flavonoid, which may function as a potential chemopreventive and chemotherapeutic agent. Our previous data reported that down-modulation of PKCε level caused by zapotin was associated with decreased migration and increased apoptosis in a cancer cell line (HeLa) containing doxycycline-inducible constitutively active PKC epsilon (PKCεA/E, Ala159 is replaced by Glu). Moreover, we showed decreased levels of Bcl-2, PARP-1, c-Jun, c-Fos and NFκB. The observed effects were dependent on the zapotin concentration.

Because autophagy may either precede apoptosis or be simultaneously depended on the genes expression and metabolic status of cells, we studied the effect of zapotin on the autophagy process in cancer cells with overexpressed PKCε. We revealed that increasing concentration of zapotin caused inhibition of autophagy. Decreased formation of autophagosomes was accompanied by a decreased level of LC3 protein. Moreover, RT-PCR analysis showed that, zapotin (30 μM) caused downregulation of *PI3KC3, BECN1, ATG5, ATG12, MAP1LC3*β and *BCL2* expression levels and upregulation of *RPTOR* and *BAX*. This was accompanied by a decrease levels of proteins PI3-K-III, Beclin1 and Atg5, and an increase amount of mTOR.

Conclusion: Our results suggest that both the anti-autophagic and the pro-apoptosis effects of zapotin in HeLaPKCεA/E cells is associated with the protein kinase C epsilon signaling pathway. Zapotin, as a natural compound, may become a potential candidate specifically activating the crosstalk between autophagy and apoptosis in cancer cells with overexpressed PKCε and can lead to programmed cell death.

Key words: apoptosis; autophagy; cancer; PKCε; flavones; zapotin

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