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Inhibition of melanoma cell growth and angiogenesis by *Punica granatum* peel extract

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Aim: It has been shown that *Punica granatum* has several anticancer actions. This study was designed to examine the *in vitro* antiproliferative effect of the pomegranate peel extract (PPE) on B16F10 melanoma cells as a carcinoma cell line. Additionally, we have investigated the in vitro effect of PPE on Human umbilical vein endothelial cells (HUVECs) angiogenesis.

Materials and Methods: Antiproliferative effect of PPE on melanoma cells and HUVECs was evaluated by MTT assay (PPE(10-450 µg/ml)); Angiogenesis have been investigated by matrigel assay (PPE(200,300,400 µg/ml)); VEGF mRNA expression was detected by RT-PCR assay and VEGF concentration in culture medium of HUVECs was determined by ELISA.

Results: Incubation of melanoma cells with PPE for 48 h caused reduction in cell survival. The matrigel assay results indicated that PPE was significantly effective in preventing tube formation .We found a significant dose dependent decrease of VEGF mRNA expression and VEGF concentration in culture medium of PPE treated HUVECs ($P<0.05$). Conclusion. These results showed that PPE is effective in both suppression of angiogenesis and growth of melanoma cells *in vitro*. Further studies in *in vivo* cancer models are needed to confirm these results and find out the other effects of this extract on cancer.

Keywords: Punica granatum, Human Umbilical Vein Endothelial Cells, angiogenesis, B16F10 melanoma.

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A novel STAT3-targeting immunotherapeutic approach to cancer treatments using protein-transduction technology

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Protein-transduction domains (PTDs) are short stretches of cationic amino acids that enable peptides, proteins, and other reagents to efficiently enter multiple cell types *in vitro* as well as *in vivo*. Among the PTDs, poly-arginine peptides, especially nona-arginine (R9), is transported most efficiently with minimal cytotoxicity. Therefore, *in vivo* administration of R9-PTD-containing fusion proteins to the solid tumor masses might be applicable as a novel molecular-targeting strategy. Recent studies have identified a protein, signal transducer and activator of transcription 3 (STAT3), as an important molecule that mediates tumor-induced immunosuppression at many levels. To inhibit the STAT3-mediated signaling cascades in cancer microenvironment, we generated a fusion protein, designated rR9-GRIM19 since (endogenous) GRIM-19 molecule physically interacts with STAT3 and inhibit STAT3-dependent signal transduction. In vitro analysis of the expression of downstream molecules of STAT3 confirmed that rR9-GRIM19 treatment of murine cancer cells expressing constitutively activated STAT3 (STAT3c) significantly reduced STAT3-dependent transcription. Moreover, intratumoral (i.t.) injections of rR9-GRIM19 significantly suppressed tumor growth in cancer-bearing mice with STAT3c (A20, B16), but not in cancer-bearing mice with inactive STAT3 (EG.7). Interestingly, our recent results demonstrated that rR9-GRIM19-mediated antitumor effects were not observed in cancer-bearing nude mice, but those were restored simply by the adoptive transfer of normal CD8+ T cells with rapid T cell conversion of IL-10-producing into IL-17/ IFN-gamma-producing phenotypes. These results indicated that local administration of rR9-GRIM19 had some advantages as a novel anti-STAT3 cancer immunotherapy because it strongly inhibited STAT3-regulated molecules, including IL-10, without inducing major systemic side effects.

Biography

Naotaka Shibagaki is Associate Professor at Department of Dermatology in University of Yamanashi Faculty of Medicine. He graduated from the Yamanashi Medical College, and received his M.D. degree in 1988. He worked for Department of Dermatology in Yamanashi Medical College as Graduate Student. In 1991, he served as Postdoctoral Fellow at Department of Dermatology Emory University, U.S.A. He received his Ph.D. in 1994 at University of Yamanashi Graduate School of Medicine. In 1999, he served as Visiting Fellow under Mark C. Udey at Dermatology Branch, National Cancer Institute, U.S.A. His current research is focused on skin/ cancer immunology.