The Anti-angiogenic Effect of Soybean as a Chemopreventive Agent on Oral Carcinoma Cell Line

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Abbreviations	*Words*
HNSCC	Head and neck squamous cell cancers
OSCC	Oral squamous cell carcinoma
ROS	Reactive oxygen species
VEGF	Vascular endothelial growth factor
b FGF	Basic fibroblast growth factor
TNFα	Tumor necrosis factor- alpha
IL-8	Interleukin 8
mM	Millimole
NSAIDs	Non steroidal anti inflammatory drugs
EGCG	Epigallocatechin- 3-gallate
COX-2	Cyclooxygenase-2
DMBA	7.12-dimethylbenz[a]anthracene
HBP	hamster buccal pouch
cdc2	cell division cycle gene 2
MMP-2	Matrixmetalloproteinase-2
MMP-9	Matrixmetalloproteinase-9
UPA	urokinase plasminogen activator

CVD	cardiovascular disease
LDL	low density lipoproteins
HDL	High density lipoproteins
Bax	Bcl-2-associated X protein
Bcl-2	B-cell follicular lymphoma/ leukemia-2
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
TGF-beta	Transforming growth factor-beta
HEPA	High efficiency particle
EDTA	Ethylenediaminetetraacetic acid
HLA	Human leukocyte antigen
NSCLC	Non-small cell lung carcinoma
ATCC	American Type Culture Collection
NCI	National Cancer Institute
ARC	Agricultural Research Centre
DMEM	Dulbeco's Modified Eagle's Medium
FBS	Fetal bovine serum
MTT	(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)
TBS	Tris Buffered Saline
TTBS	Tween Tris Buffered Saline
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid

Abstract

Soybean has attracted much attention because of its health benefits and anticancer activities which include antiangiogenic properties. Phytochemicals-mediated antiangiogenic intervention has been reported to be an effective cancer prevention strategy. The present study was designed to investigate the anticancer effect of soybean on oral squamous cell carcinoma cell line (Scc-27) with respect to angiogenesis. Mtt viability assay was utilized as a preliminary test to investigate the effect of soy on the viability of cells and to determine the appropriate dose and duration that would be used for the dot blot assay. For angiogenesis assessment, the dot blot assay was used to determine the effect of soy extract on the expression of VEGF immunochemically using different doses of soy extract and different time intervals for its application. Soy extract was found to increase the viability of cells in a dose and duration dependent manner. The dot blot assay revealed a significant inhibition of VEGF expression as the dose of soy extract and its time of application increased. The current study concluded that soy bean is a natural chemopreventive agent that has an anticancer effect on oral squamous cell carcinoma with respect to angiogenesis.

Introduction and Review of literature

Carcinogenesis is a multistep process at both the phenotypic and the genetic levels, resulting from the accumulation of multiple mutations. Fundamental to the origin of all neoplasms are genetic changes (mutations) that allow excessive and unregulated proliferation that is independent of physiologic growth-regulatory stimuli. Neoplastic cells are said to be transformed because they continue to replicate, apparently independant to the regulatory influences that control normal growth. Therefore, cancer is not one disease but many disorders that share a profound growth dysregulation ***(Kumar and Robbins 2007)***.

Studies of the origins of cancer have shown that a combination of genetic influences and environmental causes over time triggers gene mutations, which may explain why most cancers are seen in adults of middle age or older. Most cancer-related deaths are due to tumor involvement of critical organs or to complications in therapies aimed at controlling tumor growth and spread ***(Janes-Hodder and Keene 2002 & Roomi et al., 2009)***.

Head and neck squamous cell cancers (HNSCC) are the sixth most common cancers in the world and are a major cause of significant morbidity ***(Jemal et al., 2004)***. Oropharyngeal cancer is a cancer that develops in the oropharynx including the base of the tongue, soft palate, tonsils, tonsillar pillars and the side/back

walls of the throat and it is one of the 10 most common types of cancer worldwide ***(Davidson and Touger-Decker 2009)***.

Oral squamous cell carcinoma (OSCC) is the most common of the head and neck cancers. Multiple primary tumors are a known phenomenon and local–regional recurrence is a common and challenging oncological problem in patients affected by this disease ***(Yang et al., 2006)***. Squamous cell carcinoma (SCC) may develop through a progressive sequence from mild to severe dysplasia, carcinoma in situ and, finally, invasive carcinoma. The tumors present as fungating, ulcerating or infiltrating lesions ***(Stoner et al., 2007a)***.

There are several factors involved in the etiology of SCC which include excessive use of tobacco, alcohol consumption, consumption of salt-cured and salt-pickled food, vitamin and trace minerals deficiencies, radiation therapy and human papilloma virus ***(Stoner et al., 2007a)***.

Environmental carcinogens such as nitrosamines found in tobacco are able to affect the genetic material of host cells inducing aberrant regulation of multiple genes in cells leading to uncontrolled growth and, ultimately, cancer ***(Stoner et al., 2007a)***.