2. ABSTRACT OF THE THESIS

Cancer is a complex disease which can be attributed to accumulation of several genetic alterations resulting in malignant transformation of normal cells into highly cancerous derivatives. Genetic alterations in cancer cells are regulated by several oncogenes or tumor suppressor genes which modulate tumor microenvironment as well as various cellular phenomenon governing tumor progression. Therefore, cancer biologists are currently keen to understand the possible role of various tumor suppressor genes in regulation of tumor progression. Semaphorins constitute a family of secreted, membrane bound molecules which serve as guidance clues during embryonic development. Sema 3A, originally identified as chemorepellent during axonal guidance is known as a candidate tumor suppressor which attenuates breast tumor progression. Sema 3A binds with Neuropilin-1(NRP-1) which couples with plexin A1 to serve as the signal transducing unit for Sema 3A/NRP-1 complex. However, the underlying mechanism by which Sema 3A suppresses breast tumor growth is not clearly understood. Here, we report that Sema 3A regulates PTEN and FOXO 3a nuclear translocation. Moreover, Sema 3A-induced FOXO 3a activation is dependent on NRP-1 and mediated by PTEN. Overexpression of PTEN and FOXO 3a attenuates Sema 3A-mediated breast cancer cell migration. Chip assay data revealed that FOXO 3a binding site is present on MelCAM promoter. Furthermore, Sema 3A induces NRP-1-mediated MelCAM expression and PTEN, FOXO 3a regulate this process. Loss of or gain in function data revealed that Sema 3A regulates phosphorylation and expression of PTEN, FOXO 3a and MelCAM in in vivo mice model. Clinical specimen analysis revealed that expression of Sema 3A and p-PTEN are correlated with breast cancer progression, further strengthening our in vitro and in vivo findings. We for the first time established a functional correlation between chains of tumor suppressor genes with attenuation of breast cancer progression.

Sulforaphane (SFN; 1-isothiocyanato-4-(methylsulfinyl)-butane), a naturally occurring phyto-chemical compound has recently drawn attention of cancer biologists owing to its anti-carcinogenic properties and clinical significance. SFN acts synergistically with other anti-cancer compounds or tumor suppressors to enhance their anti-tumor activities. Sema 3A, a well known tumor suppressor of class 3 semaphorin family is also known to induce apoptosis in neuronal cells. We,
for the first time have elucidated an apoptotic signaling mechanism where Sema 3A triggers anti-cancer activity of SFN when used in combination in breast cancer cells. Sema 3A in combination with SFN significantly reduced the cell viability and motility of breast cancer cells. Treatment of both Sema 3A and SFN resulted in decreased expression of anti-apoptotic molecules and simultaneously enhanced the expression of pro-apoptotic molecules. Sema 3A and SFN-induced apoptosis is regulated by Akt pathway. Sema 3A in combination with SFN significantly inhibits ERK activation without having any significant effect on p38 activation. Thus, apoptotic response induced by Sema 3A in presence of SFN is through downregulation of ERK pathway. Sema 3A in combination with SFN decreased breast tumor growth in \textit{in vivo} mice model. Western blot and immunohistochemical studies of the mice sections further confirmed that Sema 3A and SFN-induced apoptotic response is through regulation of Akt and MAPK pathways. Thus, this novel combination of a tumor suppressor, Sema 3A and a natural compound, SFN could serve as a vital therapeutic strategy for treatment of breast cancer.