3. REVIEW OF LITERATURE

a. Nrf2 is a key regulator of cell proliferation and redox status of a cell:

Expression of Nrf2 in cancer cells promotes cell proliferation and inhibits apoptosis by activating PI3K-Akt signaling pathway (50, 67, 114). Activated PI3K-Akt pathway in turn augments the nuclear accumulation of Nrf2 there by further enhances cancer cell proliferation (115, 116). In addition, this Nrf2-Akt activation-reactivation loop leads to re-direction of glucose into the anabolic pathway (to increase metabolism) indicating the reinforcement of metabolic reprogramming by Nrf2 (37, 58, 117-119). A recent study by DeNicola, G.M., 2015 also showed an important role for Nrf2 in metabolism. Results of this study demonstrated that Nrf2 regulates serine / glycine biosynthesis in non-small lung cancer cells by controlling the expression of key enzyme genes PHGDH, PSAT1 and SHMT2 via ATF4 to support glutathione and nucleotide production. Prior studies have implicated these enzymes in poor prognosis of human NSCLC (119).

Furthermore, many prior studies by Tian Y, et al. (2014), Chen XL, et al (2004), Nguyen T et al (2009), Castaldo SA, et al (2016), Yadetie F, et al (2012) have shown that activation of Nrf2 results in the induction of many cytoprotective proteins that include: (A) Intracellular redox balancing proteins such as glutamate cysteine ligase (GCL), glutathione peroxidase (GPx), thioredoxin (Trx), thioredoxinreductase (TrxR), and peroxiredoxin (Prx), and heme oxygenase-1 (HMOX-1); (B) Phase-II detoxifying enzymes that include glutathione S-transferase (GST), NAD(P)H quinone oxidoreductase-1 (NQO1), and UDP-glucuronosyltransferase (UGT); and (C) Transporter Proteins comprising of multidrug resistance-associated protein (MRP) (36, 120-127). Furthermore, a recent study measuring the expression of Nrf2 targets has identified about 100 genes to be the transcriptional targets that are involved in inflammation and cancer development (128, 129).

b. Nrf2 activity is enhanced by mutations in its negative regulator Keap1 in lung cancers:

In lung cancers Nrf2 and its negative regulator Keap1 are often mutated, which lead to enhanced cell proliferation, drug and radio-resistance (40, 69, 130, 131). However, the potential of mutational status to predict Nrf2 activity is not well established. Addressing this, a recent study by McLeod, A.K. et al., 2016 reported that four members of the aldo-keto-reductase (AKR) super family were heavily expressed in cell lines and tumor biopsies whenever there is a mutation in Nrf2 pathway (132). Therefore AKRs could serve as potential markers for Nrf2 mutation prediction (132-135). Mutations in Nrf2 are primarily somatic and occur in the Neh2 domain of Nrf2 in adenocarcinoma and Squamous cell carcinoma (SCC) lung tumors (136-139). Nrf2 with these mutations fails to dimerize with MAF proteins as well as DNA binding to ARE regions (70, 140). For example, mutations within the DLG (amino acid 27-32) and ETGE (amino acid 77-82) regions affect the binding affinity to the KELCH domain of KEAP1 and inhibit redox-sensitive repression by KEAP1, which normally controls the basal levels of NRF2 expression (70, 141, 142). The inability to maintain NRF2 protein expression at or below a basal level causes lung adenocarcinomas and squamous cell carcinomas (40, 143-145).
c. Elevated Nrf2 induces drug resistance in cancer cells:

Elevated NRF2 expression induces resistance to specific anticancer chemotherapeutics as observed in human cancer cell lines A549 (very high Nrf2), NCI-H292 (mucoepidermoid cells, moderate Nrf2), and RERF-LC-Ai (SCC cells, very low Nrf2). While A549 with very high Nrf2 showed strong resistance to cisplatin, the NCI-H292 and RERF LC-Ai cells exhibited moderate and low resistance as they have medium and low levels of Nrf2 expression (48, 146) (147, 148). Hence, the expression of Nrf2 is proportional to the level of drug resistance to cisplatin. Further analysis of key proteins involved in antioxidant activity, phase II metabolic enzymes, and drug efflux pumps also showed similar elevated expression pattern, which is in proportion to NRF2 (57, 149). For instance multidrug resistance protein 3 (MDR3), a known drug resistance protein in NSCLC, is directly induced by NRF2 in NSCLC tumor tissues as well as in cell lines representing carcinomas of prostate (DU-145), and lung (H1666, H1650, and A549) (70, 150, 151). Likewise, a recent study by Mine, N et al., 2014 showed activation of Nrf2 in CBP501 (a peptide based anticancer drug tested in Phaes-II clinical trials) resistant NSCLC cells compared to CBP501 sensitive cells (152). In summary, Nrf2 is a good marker for patient treatment decisions as well as for monitoring the drug efficacy in non-small-cell lung cancer.

d. Nrf2 activators are potential chemopreventive agents:

While many studies like the ones mentioned above shows the oncogenic potential of Nrf2 and its pivotal function in drug resistance induction, few recent studies have provided strong evidences to demonstrate that expression of functionally active Nrf2 helps to mitigate the transformation of normal cells in to cancer cells (52). Hence, pharmacological agents upregulating Nrf2 could be considered as better chemopreventive agents (52, 153, 154). A recent investigation addressing whether pharmacological activation of Nrf2 provides any survival advantage to cancer cells showed that targeted activation of Nrf2 using antioxidant inflammatory modulator (AIM) compounds such as RTA405 will not activate survival kinases BCl2 and IKKβ in cell lines harboring a wild type Keap1 protein (155). Hence, treatment of cancer cells with Nrf2 activating pharmacological agents will not provide any survival advantage to these cells (58, 107, 156).

In a separating finding, Shen, T et al., 2015 demonstrated that a derivative of curcumin, bis [2-hydroxybenzylidene]acetone (BHBA), inhibited vinyl carbamate induced lung tumorigenesis by upregulating Nrf2 and its downstream target genes NQO1 and GCLM (157). Likewise, another anticancer agent sphingosine kinase-II inhibitor (SKI-II), also reported to activate Nrf2 by promoting the dimerization of Keap-1 thereby releasing the Nrf2 (158). Furthermore, many recent evidences from epidemiological and preclinical studies continues to point that phytochemicals such as sulforaphane (SFN), phenylethyl isothiocyanate (PEITC), Diallyl sulfide, and epigallocatechin-3-gallate (EGCG) activate Nrf2 thereby prevents the transformation of normal cells in to cancer cells by increasing the detoxifying effect on excess reactive oxygen species (ROS)(71, 159, 160).

Therefore, modulating Nrf2 expression and activity is an important strategy to control cancer cell growth. Whereas Nrf2-activators serve as better chemopreventive agents, the inhibitors of Nrf2 act as potent chemotherapeutic agents. Hence, in this study phytochemicals were isolated (partially) from reliable sources and tested for their ability to inhibit cancer growth.