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Can Butein be a Future Candidate for the Treatment of Advance Metastatic Thyroid Cancer?

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The incidence and prevalence of papillary thyroid cancer (PTC) are increasing worldwide and it is the 5th most common endocrine cancer in females [1]. In addition to this, the frequency of resistance toward radio-iodine therapy is also increasing in PTC patients (Advanced metastatic thyroid cancer). External beam radiation therapy (EBRT) and chemotherapy are used for the treatment of such patients. EBRT and Chemotherapy are associated with serious side effects and toxicity. US-FDA has also approved two drugs (Sorafenib and Lenvatinib) for the treatment of advanced thyroid cancer patients. However, the efficacy of both drugs is limited in terms of overall survival and disease-free survival and associated with severe toxicities [2]. Hence, the treatment of patients with advanced metastatic thyroid cancer represents a major challenge for clinicians and oncologists. In such patients, tumor cells show invasion in local neck regions, lungs, and bones [3]. Metastasis is the most dangerous aspect of cancer and is responsible for 90% of deaths of cancer. Epithelial -mesenchymal transition (EMT) and cancer stem cells (CSC) are the driving forces of metastasis and therapeutic resistance [4]. Hence, this axis of EMT and CSCs is a major target from the new therapy point of view.

One approach for novel cancer therapeutics is to look for plant-based phytochemicals, especially flavonoids. Extensive studies were performed to explore the cytotoxic and anti-metastatic effects of flavonoids. Flavonoids may exert an anti-metastatic effect by reversing EMT or modulation of EMT governing signaling pathways. Several reviews have shown that different subgroups of flavonoids reduce the expression of mesenchymal markers like vimentin, N-cadherin, matrix metalloproteinase, increase E-cadherin expression, and target CSCs [5,6]. Chalcones, α, and β unsaturated ketones, are one of the most important bioactive flavonoids with therapeutic potential linked to a variety of biological effects studied in various *in-vitro* and *in-vivo* investigations [7]. From ancient times plant-derived chalcones especially butein is used for the treatment of several diseases. Butein is extracted from *Butea-monosperma, Rhus-verniciflua*, and *Semecarpus- anacardium*. It has been reported to have antimicrobial, antioxidant, antiviral, anti-inflammatory, cytotoxic, anti-malarial, anti-diabetic, and vasodilatory effects and is used as herb in South East Asia region. Further, reports have also shown the anti-cancerous effect of butein in various cancer models [8]. It was also demonstrated that in thyroid cancer, butein induces an intrinsic pathway of apoptosis, proteolysis of vimentin, and inhibits cancer stem cells in the human papillary thyroid cancer cell line. Additionally, in the paper by Tripathi & Kulkarni [7], the effect of butein at low and high doses was also demonstrated. At low doses, butein induces inhibition of cell migration and cancer stem cells through suppression of enhanced glycolysis and vimentin phosphorylation. While butein induces caspase activation and downregulates anti-apoptotic protein in the NPA cell line for the execution of the intrinsic pathway of apoptosis and vimentin proteolysis at high concentration [9].

The study has further raised important questions which are discussed sequentially: to be addressed. 1) mechanism behind the low and high doses response of butein, 2) long term exposure of butein and its relation with EMT and CSCs mediated cancer resistance, 3) Butein and cancer cell metabolism with reference to EMT and CSCs, 4) Safety of butein to normal cells and 5) Probability of utilization of butein as an anti-cancer therapeutic agent for the thyroid cancer or to increase the efficacy of present therapeutic regime.

The differential dose-response is a dynamic process or mechanism and depends upon the concentration and time duration. For example- curcumin exerts anti-oxidant properties like reactive oxygen species scavenging, activation of antioxidant enzymes, anti-apoptotic proteins, and heat shock proteins at low dose while induces DNA damage, apoptosis and pro-oxidant property at high dose. One of the mechanisms of the differential response of curcumin based on metabolic profiling of MCF-7 and MDA-MB-231 is the biphasic levels of glutathione in cancer cells (high level of glutathione at low dose and low level of glutathione at high dose) [10]. Butein also increases total GSH levels at 5, 10 and 25 μM in hepatocytes and protects them from tert-butylhydroperoxide (tBHP) mediated oxidative stress through ERK/Nrf2 pathway [11]. Similarly, butein increases heme oxygenase activity through Nrf2/ARE pathway to prevent H_2O_2 induced cell death in dental pulpal cells at 2.5, 5, 10 and 20 μM concentrations [12]. Butein may influence the redox status of cells in a differential way depending upon the dose and time duration. Hence, it is important to explore and decipher the effect of different doses of butein in suitable thyroid cancer models for the assessment of redox status, cell-protective signaling pathways (ARE/ Nrf2 pathways), and cell-protective proteins to further understand the mechanism of action at low and high doses of butein.

Long-term exposure to chemotherapeutic agents especially at low doses is associated with the development of resistant clones in cancer cells which finally leads to decreased response toward standard therapies [13]. Studies have further shown that exposure to chemotherapy leads to EMT activation and an increase in CSCs [14,15]. EMT and CSCs axis is the major reason behind the metastasis, recurrence, and therapeutic resistance [16]. EMT plays an important role in the acquisition of mesenchymal characteristics like vimentin and CSCs emergence. In PTC, vimentin is linked with invasion, migration, and poor prognosis [17]. In directional migration, vimentin interacts with microtubules and actin filaments and co-ordinate their dynamics. Microtubules contribute to the polarity in the migratory cells and Microtubules have a shorter life in comparison to vimentin. During cell migration, vimentin disassembles from the periphery and undergoes retrograde transport to incorporate into the mature filaments. This retrograde flow of vimentin helps in restricting actin retrograde flow and prevents nucleus collapse. Disassembly of vimentin from the periphery of the cell act as template for the growing microtubules and maintaining the cell polarity. Thus, vimentin acts as a template and guide the growing microtubules for the maintenance of cell polarity [18,19]. In a cancer thyroid cell, Merca cell line (A murine cell line developed from-Braf V600E mice), ectopic expression of Snail (EMT transcription factor) induces in vimentin and CSCs upregulation. This induction of EMT and stemness was significantly inhibited by Celastrol a natural inhibitor of neoplastic cells [20].

Vimentin's role in cancer depends upon the structure and post-translational modification (PTM) status of the vimentin. Phosphorylation of vimentin is the most important PTM. Eriksson et al. characterized the ^{32}P labeled phosphovimentin filament and showed the critical serine and threonine residues are important for phosphorylation. They further reported in BHK-1 fibroblast cells that protein phosphatase inhibition with calyculin A resulted in fast vimentin phosphorylation and phosphorylation induced disassembly into the soluble tetrameric vimentin oligomer [21]. Similarly, in serum-starved fibroblast, vimentin filaments were shown to get assembled in the periphery region. Activation of Rac1 in serum-starved fibroblast phosphorylates vimentin at the ser-38 residue. Phosphorylation induces depolymerization and retraction of vimentin from the cell surface where lamellipodia formation take place [22]. Various protein kinases like PKA, PKB, PKC, p21 activated kinase, Aurora B, RhoA-binding kinase α, and CaMkII; phosphorylate vimentin protein at various serine residues and influence the assembly of the vimentin filaments [21,23]. Ivaska et al. had shown that PKC mediated vimentin phosphorylation is important for the integrin trafficking and cell migration towards the matrix [24]. Hence, vimentin is suitable target for the development of an effective anti-metastatic agent.

Our study demonstrated that Butein induces inhibition of vimentin phosphorylation and further induces caspase -mediated proteolysis in the NPA cell line [7]. Zhu et al., had shown that protein kinase B (Akt) directly interacts with vimentin and induces serine 39 phosphorylation. Inhibition of vimentin phosphorylation or substitution of serine with alanine not only suppresses cancer cell migration but also induces caspase-mediated proteolysis [25]. Further, vimentin is important for cell division and stemness. A small molecule like FiVe1 binds with vimentin and targets stemness in mesenchymal cancer cells [26]. Thus, the development of small molecule inhibitors which directly target the activity of vimentin is a good approach for the development of anti-metastatic therapeutic agents. Hence, it is important to study the interaction between vimentin and butein to decipher the mechanism of the anti-metastatic effect. Butein may directly interact with vimentin domains to inhibit its phosphorylation or butein may inhibit activities of several protein kinases for the inhibition of vimentin phosphorylation. However, *in silico* and *in vitro* studies are required to establish the interaction between vimentin and butein in more depth to decipher the mechanism of butein at low and high concentrations in more kinetic and structural approach.

An altered metabolism plays an important role in cancer cell proliferation, migration, invasion, EMT, drug resistance and CSC maintenance. Cancer cells displayed altered glucose metabolism, glutamine metabolism and also changes the regulation of key enzymes for the metastasis, drug resistance and CSCs [27,28]. Several oncogenes play an important role in cancer metabolism. In thyroid cancer, epidermal growth factor receptor (EGFR) alternation is linked with dedifferentiation, EMT and metastasis [29]. EGFR signaling also influence various metabolic functions like glucose utilization to fatty

acids and nucleotide synthesis [30]. Hence, it is important to inspect effect of butein on cancer cell metabolism and genetic alternations. In hepatocellular carcinoma, butein inhibits EGFR and hexokinase-2 interaction to inhibit the glycolysis and proliferation [31]. Similarly, butein inhibits the glucose induced cell proliferation, induces ROS, DNA damage and p38 activation to induce anti-cancerous effect on non-small lung cancer cells [32]. Our study on NPA cell line also showed that butein inhibits glycolysis at low concentration [9]. Hence, it is important to investigate effect of butein on metabolic changes, EGFR and its link with genetic changes in thyroid cancer to reveal the mechanism through metabolomics approach.

Activation of EMT leads to the process of dedifferentiation in cancer cells. Dedifferentiation of thyroid cancer leads to loss of sodium iodide transporter and decreased radioiodine uptake [33,34]. Hence, long-term exposure to butein should be explored in suitable thyroid cancer models especially in radioiodine concentrating and radioiodine non-concentrating cells for evaluation of EMT markers and CSCs. This type of study also will reveal the effect of butein on iodine uptake and redifferentiation in advanced metastatic thyroid cancer cells. It was already known that quercetin treatment increases NIS expression in NPA cells and in anaplastic thyroid cancer cells, iodine-131 labeled quercetin was accumulated more in comparison to iodine 131 alone [35,36]. For long-term exposure studies, it is also important to study the effect of butein when used in combination with EBRT and chemotherapy to increase the efficacy of these therapies.

The most important aspect of any novel chemotherapeutics is not only the anti-cancerous activity but also its toxicity towards normal cells. Chemotherapy, radiotherapy, and various protein receptors or protein kinase inhibitors are associated with strong side effects. Hence, toxicological aspects of butein are important from a therapy point of view. Toxicological studies have shown that butein is safe for the normal cells and an oral administration of butein into the rats was found to be safe. Butein had no cytotoxicity towards monocytes, normal lymphocytes, and oral cells like periodontal ligament cells, pulp cells, and gingival fibroblast cells [37]. However, its effects on normal thyrocytes, hormone synthesis by thyroid, and its metabolism are not known. Hence, the effect of butein needs to be investigated in a proper animal thyroid model to explore the effect on the normal thyroid gland.

In summary, the progression and promotion of resistance to therapies, resulting from EMT activation and CSCs emergence has led to the importance of EMT and CSCs promoting signaling pathways in thyroid cancer. Further investigation in the mechanisms by which butein inhibits EMT and CSCs in thyroid cancer will contribute to the mechanistic aspect involved especially when the use of butein is considered as a thyroid cancer therapeutic agent.

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