Aloysia Citrodora Essential Oil

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Patients diagnosed with melanoma have a poor prognosis due to regional invasion and metastases. The receptor tyrosine kinase epidermal growth factor receptor (EGFR) is found in a subtype of melanoma with a poor prognosis and contributes to drug resistance. Aloysia citrodora essential oil (ALOC-EO) possesses an antitumor effect. Understanding signaling pathways that contribute to the antitumor of ALOC-EO is important to identify novel tumor types that can be targeted by ALOC-EO.

Keywords: melanoma ; EGFR ; MMP ; Aloysia citrodora ; HB-EGF ; plant ; proliferation ; metastasis ; ERK1/2 ; herb

1. Introduction

Cutaneous melanoma is an aggressive tumor with increasing incidence worldwide. B-Raf has a mutation rate of up to 90% in melanoma [1][2][3]. Despite major efforts to improve treatment, including the introduction of signal transduction inhibitors like B-Raf enzyme inhibitor and MEK inhibitor or immune checkpoint blocker, no significant advances in patient survival have been obtained in the last two decades in patients with advanced disease [4][5][6][7]. One problem in treating patients is that tumors can develop acquired resistance. Novel drugs are urgently needed.

Essential oils (EO), which are concentrated hydrophobic liquids from aromatic plants, have shown anticancer properties enabling them to penetrate the cell membrane and act on cellular targets like Akt and mTOR, and have been shown to increase apoptosis by upregulating caspases 3 and 9 [8]. Metabolites contained within EOs help plants defend themselves against herbivores, insects, and microorganisms [9][10]. A cheap alternative to conventional chemotherapeutic drugs for overcoming the occurrence of multidrug resistance, the anticancer effects of plants are being brought back into the spotlight. Aloysia citrodora (ALOC) Paláu (Lippia citriodora Kunth), also known as “lemon verbena”, is a medicinal plant native to South America, North Africa, and southern Europe, where it is used by native people to treat diarrhea, flatulence, insomnia, and rheumatism, among other maladies [11]. The ALOC EO contains chemically aromatic secondary plant metabolites like neral, geranial, limonene, and 1,8-cineole, while the extract contains verbascoside derivates and flavonoids [11]. EOs from ALOC have been reported to show anticancer activities [12]. Studies by Zeng et al. showed that geranial and neral inhibited tumor growth in the 4T1 breast cancer xenograft mouse model [13].

Autocrine/paracrine production of epidermal growth factor receptor (EGFR) ligands, like epiregulin, amphiregulin, and heparin-binding EGF-like growth factor (HB-EGF), and the overexpression of EGFR, are two of the mechanisms most frequently implicated in cancer development and progression. HB-EGF is involved in the progression of tumors like hepatocellular carcinoma, colon carcinoma, and melanoma. HB-EGF promotes melanoma growth through its interaction with EGFR and in its role as a MAPK and PI3K/Akt pathway activator [14][15][16]. Expression of EGFR in melanoma establishes a pro-metastatic phenotype [17] that can activate Ras/MAPK, PLCγ1/PKC, Akt, and STAT, which subsequently stimulate cell proliferation, migration, invasion, survival, and differentiation. The receptor tyrosine kinase EGFR is activated in a subset of melanoma cells [18][19] where its expression correlates with poor prognosis in human melanoma [20][21].

The expression of various receptor tyrosine kinases, like AXL, PDGFRB, and EGFR, in cutaneous melanoma occurs in $MITF^{low}$ melanoma cells with a pro-invasive potential and has been associated with BRAF/MEK inhibitor resistance. Studies using human melanoma cell lines and experimental murine melanoma models found that EGFRs are promising therapeutic targets. Bardeesy et al. demonstrated the existence of an EGF signaling loop essential for H-RASV12G-mediated tumorigenesis using an inducible transgenic mouse model [22]. EGFR gene copy number alterations and polysomy of chromosome 7—where the EGFR gene is located—are correlated with poorer prognosis in human melanoma [23]. In addition, EGFR expression is higher in metastases compared with primary tumors [24].

Metalloproteinase-7 (MMP7) expression was demonstrated in the majority of primary tumors and all metastatic tumors in melanomas and therefore was proposed as a prognostic marker [25]. MMP-9 (gelatinase B), similar to MMP-2, is present

in melanoma both in tumor cells and stroma [26]. A disintegrin and metalloproteinase domain-containing protein 9 (ADAM9) is expressed in human melanoma at the tumor-stroma border, where direct or indirect interactions between tumor cells and fibroblasts occur [27].

2. Current Insights

Despite recent advances in the molecular, pathological, and biological understanding of melanoma, melanoma remains a devastating disease, especially in advanced disease stages due to drug resistance, demonstrating the urgency to identify novel effective treatments.

Analysis of the effects of ALOC-EO on primary melanoma growth. We found that ALOC-EO inhibited the growth of skin cancer including melanoma cell lines in vitro, and showed its anti-proliferative effect using a murine melanoma model. Mechanistically, ALOC-EO inhibited EGFR signaling and prevented ligand (HB-EGF)-mediated melanoma cell proliferation. Our results showed that ALOC-EO blocked ERK1/2 phosphorylation and increased Bax in ALOC-EO-treated melanoma cells. Our data are in line with a study by Caudhary et al., who reported that the ALOC-EO compound geraniol inhibited the number of tumors in a murine skin tumorigenesis model by modulating cyclooxygenase-2 expression, Ras-ERK1/2 signaling, and upregulation of the pro-apoptotic BAX protein [28].

In cancer including melanoma, MMPs like MMP2/7/9 facilitate invasion/metastasis and participate as regulators of tumor cell proliferation and apoptosis [29][30][31][32]. These proteases degrade collagen type IV which is the major component of the basement membrane [33]. Earlier studies by Meierjohann et al. demonstrated that MMPs promoted melanoma migration and proliferation [34]. It was shown that EGFR signaling regulates both invadopodia formation and ECM degradation [35], and that EGFR inhibitors impaired MMP expression in melanoma cells [36]. In line with these reports, we could show that EGFR signaling blockade in part prevented MMP7/9 and ADAM9 expression. Given that these proteases are also important sheddases for HB-EGF, one of the main EGFR ligands, future studies should evaluate the potential of ALOC-EO to block MMP activation rather than transcriptional regulation.

Drugs inhibiting the activity of ERK1/ERK2 and downregulating the expression of MMP-9 can reduce invasiveness [37][38]. We showed that ALOC-EO suppressed ERK1/2 expression. This is of interest, as ERK1/2 mediates MAPK signals to cytoplasmatic and nuclear effectors. The anti-proliferative effect of geraniol, one of the compounds in ALOC-EO, was shown to reduce ERK1/2 expression on human colorectal adenocarcinoma Caco-2 cells and could be a potential candidate mediating the anti-melanoma effects observed with ALOC-EO in this study. Further studies will be necessary to test those compounds for melanoma cells. Another ALOC-EO compound is citral, which has been shown to block tumor progression in a two-stage skin-carcinogenesis model. Our results of the anti-EGFR effects of ALOC-EO are further substantiated by others demonstrating that ALOC-EO-treated fibroblasts show a decreased expression of tissue remodeling biomarkers collagen- and -III, plasminogen activator inhibitor-1 (PAI-1), and EGFR. Further studies will be necessary to determine which of the ALOC-EO compound(s) mediate the blockade of the HB-EGF-EGFR pathway in melanoma cells. We recently found that PAI-1 controls post-surgical adhesion through the EGF-HER1 axis. It will be interesting to demonstrate the potential of ALOC-EO for the treatment of postoperative adhesion after surgery [39].

A recent study demonstrated that stress-induced NRF2 binds to the ARE in the EGF promoter and leads to elevation of soluble EGF, while simultaneously blocking MITF activity, resulting in derepression of EGFR and TGFA [19]. This leads to EGFR activation. It will be interesting to determine whether ALOC-EC or its components modulate NRF2 expression.

Anti-EGFR monoclonal antibodies have been approved for the treatment of metastatic colorectal cancer [40] and head and neck cancer [41]. Erlotinib, which is an EGFR tyrosine kinase inhibitor, is FDA-approved for advanced/metastatic lung cancer [42]. However, the anti-EGFR targeting antibody Cetuximab could not influence the overall survival as shown in a study of second-line treatment of colorectal cancer [43].

Myelosuppressive drugs put therapeutic pressure on tumor cells, resulting in the disappearance of tumor clones or the upregulation of pro-survival signals that drive polyclonal-acquired drug resistance [44]. Melanoma or non-small cancer cells, among other cancer cells, acquire drug resistance by upregulating EGFR after BRAF exposure. Receptor tyrosine kinase signaling including HB-EGF-EGFR is upregulated in human SKmel-28 cells during the gain of resistance [45]. Other studies showed that six out of 16 melanoma tumors acquired EGFR expression after the development of resistance to BRAF or MEK inhibitors [46], and acquired resistance was found with the overexpression of signaling receptors like EGFR, PDGFRβ, or MET that reactivation the MAPK pathway. Following BRAF or MEK inhibitor treatment, melanoma tumors had acquired EGFR expression. Chemotherapy-induced EGFR activation is regulated by HB-EGF [47]. Accordingly, the further development of alternative agents based on EGFR signaling inhibition strategies are required to provide clinical

benefit to patients with epithelial malignancies. Here, we showed that ALOC-EO could be one such therapeutic strategy to improve drug sensitivity in melanoma cells.

Oncogenic BRAF, NRAS, NF1 mutations can activate the tyrosine kinase receptor EGFR resulting in enhanced ERK1/2 phosphorylation in melanoma. The ERK1/2 pathway communicates signals from surface receptors like EGFR into the nucleus that is also linked to Raf mutations, known to occur in up to 90% of patients with melanoma. Activation of ERK1/2 signaling is activated as a cellular defense of human non-small cancer cells against the neutralizing antibody against EGFR (cetuximab) and fractionated irradiation treatment. These studies argue that the combined targeting of EGFR and ERK1/2 might be beneficial in these cancer types. Our data demonstrate that ALOC-EO inhibited ERK1/2 in melanoma cells, and provide another argument for ALOC-EO to be used as a co-treatment together with conventional antitumor drugs.

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