INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Therapeutic Insights of Entomopathogenic Mycotoxins in Breast Cancer Cells

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SHORT ABSTRACT

Chemotherapeutic resistance in breast cancer, especially in aggressive forms like triple-negative breast cancer (TNBC), presents a major obstacle in treatment, driving the search for more effective therapeutic agents. TNBC is marked by high mortality rates and limited treatment options, underscoring an urgent need for targeted therapies. Researchers have focused on unique fungal-derived compounds with anticancer properties that may offer novel approaches to overcome drug resistance and directly targeting cancer cell survival mechanisms. Two such compounds, the ribotoxin Anisoplin and the secondary metabolite Beauvericin, have demonstrated promising effects against breast cancer, especially in TNBC, where they can potentially overcome resistance and induce cell death through unique molecular pathways.

Anisoplin, a fungal ribotoxin originally characterized for its activity against insects, has been explored for its anticancer potential. In this study, Anisoplin was successfully produced as a recombinant protein using an *Escherichia coli* BL21(DE3) expression system, followed by purification and in-depth *in silico*, biophysical, and functional characterization. Upon application to MCF-7 breast cancer cells, Anisoplin demonstrated a dose-dependent reduction in cell viability with an IC_{50} value of 4 μM. This reduction was associated with a 3.5-fold increase in intracellular reactive oxygen species (ROS) levels, indicating oxidative stress. Anisoplin further triggered mitochondrial membrane depolarization, ultimately leading to apoptosis, as confirmed by flow cytometric analysis. Furthermore, MCF-7 cells treated with Anisoplin exhibited a loss of self-renewal and clonal expansion capability, implying disruption of cell regenerative processes. Immunoblotting studies suggested that Anisoplin activated the JNK-dependent MAP kinase signaling pathway, with upregulated phospho-SAPK/JNK expression leading to a ribotoxic stress response. This JNK pathway activation was further correlated with NFκB downregulation, ultimately culminating in cell death and highlighting Anisoplin's therapeutic potential in breast cancer treatment.

In addition, Beauvericin, a secondary metabolite produced by another entomopathogenic fungi *Beauveria bassiana*, has garnered interest for its potent effects against TNBC cells, which are known for their aggressive growth, drug resistance, and metastatic propensity. Molecular docking and molecular dynamics simulations identified Beauvericin's key molecular targets, including MRP-1 (ABCC1), HDAC-1, HDAC-2, LCK, and SYK, with binding energies ranging from -90.1 to -105 kJ/mol, indicating strong interactions. Targeting these molecules enables Beauvericin to influence a wide range of cellular functions, from reversing drug resistance to disrupting oncogenic and epigenetic pathways. In TNBC cell lines MDA-MB-231 and MDA-MB-468, Beauvericin reduced cell viability,

with IC_{50} values of 4.4 μM and 3.9 μM, respectively, while also significantly increasing intracellular ROS levels by 9.0 and 7.9-fold. Beauvericin-induced oxidative stress was further linked to mitochondrial membrane depolarization, a precursor to apoptosis. Beauvericin also arrested the cell cycle at the G1 phase, impairing TNBC cells' ability to form and expand clonal colonies and spheroids, with IC_{50} values of 10.3 μM in MDA-MB-468 spheroids and 6.2 μM in MDA-MB-231 spheroids. These results underscore Beauvericin's multifaceted therapeutic potential in combating TNBC.

Another critical challenge in TNBC treatment is addressing its propensity for metastasis, largely driven by the epithelial-to-mesenchymal transition (EMT) process, which enhances cell migration and invasion capabilities. EMT plays a major role in TNBC spread to distant organs, and reversing EMT has become an essential therapeutic strategy. Beauvericin has demonstrated strong anti-metastatic effects by inhibiting EMT in TNBC cells through enhanced oxidative stress, as evidenced by elevated ROS levels and decreased mitochondrial membrane potential. In monolayer cultures of MDA-MB-231 and MDA-MB-468 cells, beauvericin exhibited an IC_{50} of 2.3 μM and reduced cell migration by 1.5 and 1.7-fold, respectively. Additionally, Beauvericin reversed EMT by upregulating the expression of the epithelial marker E-cadherin and downregulating mesenchymal markers such as N-cadherin, vimentin, Snail, Slug, and β-catenin, which are typically associated with EMT. The anti-metastatic effects of Beauvericin were further enhanced by its inhibition of the Notch signaling pathway, characterized by reduced Notch-1, Notch-3, Hes-1, and cyclin D3 expression. Furthermore, Beauvericin promoted autophagy, as indicated by the increased expression of autophagy markers LC3 and Beclin-1, which contributed to TNBC cell death and further suppression of cancer cell survival.

In summary, both Anisoplin and Beauvericin have shown significant promise as potential breast cancer therapeutics, with distinct molecular pathways involved in promoting cancer cell death and inhibiting EMT. Anisoplin induces cell death through oxidative stress and JNK-mediated apoptosis, while Beauvericin targets key molecular pathways to not only reduce TNBC cell viability but also reverse EMT and inhibit subsequent metastasis. These findings support further exploration of fungal ribotoxins and secondary metabolites as promising options in developing more effective and targeted breast cancer treatments.