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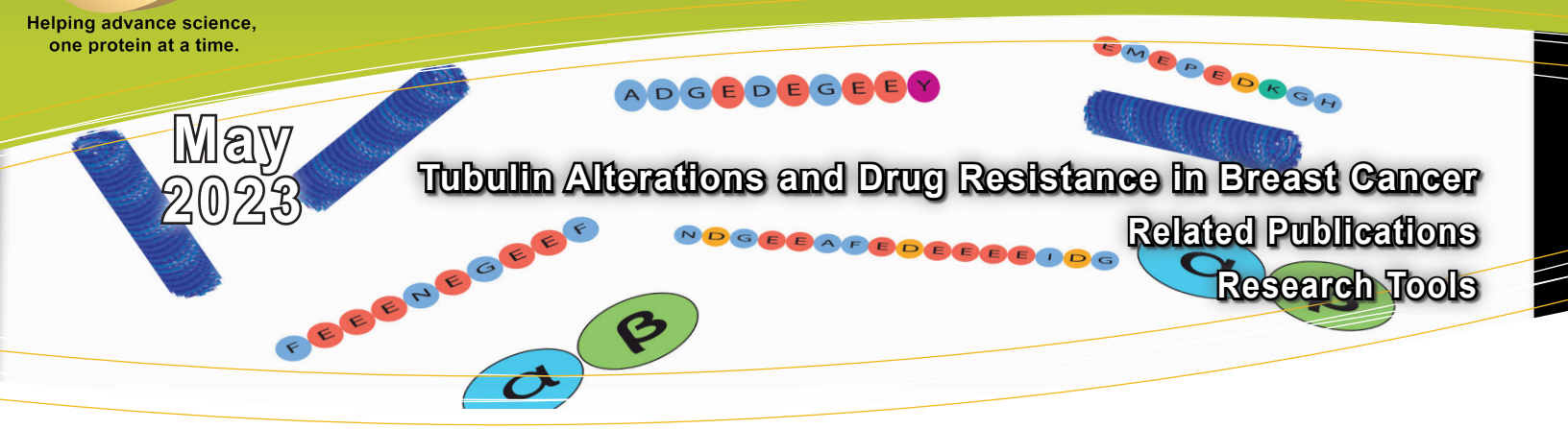

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Tubulin Alterations and Drug Resistance in Breast Cancer

Introduction

Breast cancer (BC) is currently accountable for 1 in 8 cancer diagnoses worldwide, with 2.3 million new patients annually, both sexes combined.[1] In women, it constitutes a quarter of all cancer cases and has become the most frequently detected form of the disease in 2020. This matter has been escalating globally, especially in developing countries.[2] In the United States, mortality decreased 41% since 1989; however, the descending trend has slowed, stressing the need for enhanced treatments.[3,4] During diagnosis, BC is classified according to the expression of hormone receptors (estrogen and progesterone receptors, primarily), the overexpression of the human epidermal growth factor receptor 2 (oncogene HER2),[5] and with a pathological classification that evaluates nuclear pleomorphism, mitotic count, and tubule count. Additionally, diverse alterations in the microtubule network have been detected and characterized in BC, including irregular expression of tubulin isotypes and abnormal tubulin post-translational modifications.[6] Microtubules are protein structures that constitute dynamic pillars of the cytoskeleton and have major roles in key cellular processes, such as mitotic chromosome segregation, cell shape and motility, and inner cellular transport.[7] Alterations in these essential structures in BC are associated with poor prognosis and response to treatment, and a more aggressive disease.[6]

Molecular Markers Used for Drug Response Assessment

Molecular markers expressed by cancerous cells are mutated/modified proteins that bind to hormones, gene expression patterns, and altered DNA sequences that can function as indicators of response to specific therapies.[8] The underlying cause of this phenomenon relies on DNA mutations that vary from person to person and can affect drug efficacy. Among the most well-known molecular markers related to drug resistance, there is the oncogene HER2, whose expression is amplified in 20-30% of BC cases and is regarded as a marker of poor prognosis. HER2 overexpression is linked to resistance to antihormonal and cytotoxic therapies.[8] A further example is the low transcriptional expression of CYP2D6, which predicts resistance to chemotherapy with tamoxifen (a drug that blocks estrogen stimulation) in BC.[8,9] In some cases, drug resistance has hampered the clinical success of microtubule-targeting agents (MTAs) in BC (and other cancers). More recent evidence has indicated that specific tubulin isotypes, such as class III β-tubulin, can leave microtubule drugs without effect in cells. As a result, tubulin isotypes are currently under study as potential prognostic biomarkers.[10]

Historical Use of Microtubule-Targeting Agents (MTAs) for Cancer Therapy

Given the central role of microtubules in vital cellular processes, agents that target these structures can impair normal cell function and will often lead to cell death. As cancer cells divide rapidly, they are more susceptible to cell cycle arrest-induced death; thus, MTAs have been counted among the cancer treatments of choice for decades. However, MTAs with better-targeting abilities are still sought after. The first MTA to be introduced in clinical cancer therapy was paclitaxel, which has been used for BC since 1994. Subsequent MTAs that were approved for BC treatment were the semi-synthetic taxane docetaxel, the more recent taxanes larotaxel and ixabepilone, and the vinca alkaloids.[11-14] In the present day, paclitaxel and vinca alkaloids have been established as the standard drugs in the management of different cancers, including BC.[14] Nonetheless, drug resistance due to long-term use and solubility problems have incentivized the development of novel MTAs for BC treatment, including epothilone, eribulin, auristatin, and maytansine.[15-17] The consequences of MTA therapeutic use on microtubule dynamics entail disruption of intracellular cell transport, cessation of cell division, and triggering of cell death. Noteworthy, the vast majority of MTAs interfere with microtubule functions by acting on their β-tubulin subunit.[18]

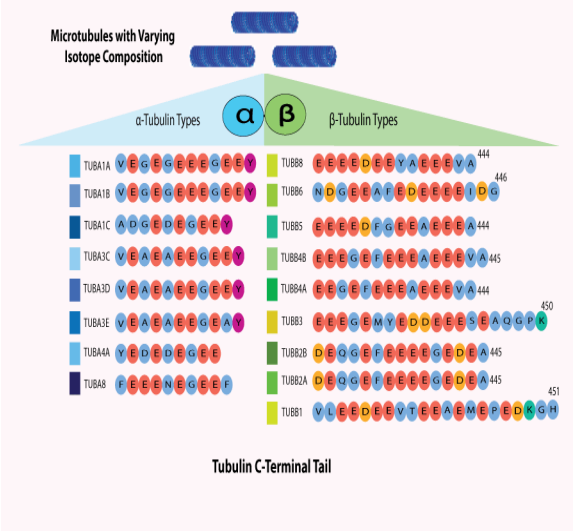

Figure 1. Schematic of alpha and beta tubulin isoptypes. Shown are the varying C-terminal sequences of these isotypes.

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Recent Findings: Alterations in βIII-tubulin Gene Expression are Associated with MTA Resistance in BC Patients

In mammals, cells are known to express not less than eight different β-tubulin isotypes, identified as βI, βIIa, βIIb, βIII, βIVa, βIVb, βV, and βVI (Figure 1).[19-20] Thorough research has evidenced that alterations in some of these isotypes in cancer cells were associated with resistance to MTAs.[10,21,22] In particular, an increased abundance of βIII-tubulin represents the most prevalent mechanism concerning MTAs resistance in various tumor types, including BC.[10,21] In this regard, Lopus et al evaluated the effects of MTA ixabepilone on BC cells with and without the *in vitro* removal and knockdown of βIII-tubulin.[23] They concluded that βIII-tubulin expression inhibits the antitumor effects of ixabepilone, denoting that increased βIII-tubulin could contribute to ixabepilone resistance. Scherbakov et al analyzed the impact of long-term incubation of ERα-positive BC cells with docetaxel.[24] For this purpose, they evaluated the expression of signaling proteins by immunoblotting and flow cytometry and assessed ERα activity via gene reporter assay. They found that the cells with the highest βIII-tubulin levels were resistant to docetaxel, while those with the lowest βIII-tubulin expression were sensitive to the drug. Extensive research in this field continues today, and further studies have claimed that βIII-tubulin does not work on its own.[25] Moreover, it has been found that the molecular pathways influenced by βIII-tubulin depend on the cell and cancer type, which could explain why some tumor types do not show poor results and resistance to MTAs at high βIII-tubulin levels.[25]

Summary and Future Perspectives

In a global context of high rates of BC, it is of utmost relevance to explore, detect, and study genetic biomarkers that can predict individual responses to the administered drugs. Overexpression of βIII-tubulin has been found in numerous cancer types, including BC, and it has been linked to poor response and resistance to various MTA. As a result, βIII-tubulin has become a considerable biomarker candidate. Nevertheless, for this knowledge to be fully exploited in clinical medicine, further conclusive research is needed, together with more studies considering the particular contexts of the patients and rigorous clinical evaluation.

Purified Tubulin

Product	Amount	Cat #
Tubulin protein (>99% pure) Source : porcine brain	1 x 1 mg 5 x 1 mg	T240-A T240-B

Tubulin Proteins

Product	Amount	Cat #
Microtubule associated protein rich fraction Source : porcine brain	1 x 100 µg 5 x 100 µg	MAPF-A MAPF-C
Tubulin protein (>99% pure) Source : porcine brain	1 x 1 mg 5 x 1 mg	T240-A T240-B
Acetylated Tubulin Protein Source: Porcine Brain Source: porcine brain	1 x 500 µg	TAC01

Tubulin Live Cell Imaging Products

Product	Ex / Em	Amount	Cat #
SiR-Tubulin™ Kit Includes SiR-Tubulin, and Verapamil	630 / 680 nm	50 nmol	CY-SC002
SiR700-Tubulin Kit 35 nmol SiR700-Tubulin and 1 µmol verapamil	680 / 720 nm	50 nmol	CY-SC014
SPY555-Tubulin Kit Includes SPY555-Tubulin and Verapamil	555 / 580 nm	100 stains	CY-SC203
SPY650-Tubulin Includes SPY650-Tubulin and Verapamil	652 / 674 nm	100 stains	CY-SC503

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Tubulin Kits

Product	Assays	Cat #
Tubulin polymerization HTS assay using >97% pure tubulin OD based - Porcine	24	BK004P
Tubulin polymerization assay using >99% pure tubulin OD based - Porcine	24-30	BK006P
Tubulin polymerization assay using >99% pure tubulin Fluorescence based	96	BK011P
Microtubule Binding Protein Spin-Down Assay Biochem Kit	50-100	BK029
Microtubule/Tubulin In Vivo Assay Biochem Kit	30-100	BK038