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Overcoming resistance

Targeting the PI3K/mTOR pathway in endocrine refractory breast cancer

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Breast cancer remains the most common cancer of women and the second most frequent cause of cancer deaths despite a major decline in breast cancer mortality in the past two decades.[1] Aberrations in molecular pathways regulating estrogen synthesis and action are primary etiological factors in the pathogenesis of breast cancer.[2] This dependence on the estrogen drive to cell proliferation and increased cell survival, together with an increased understanding of the underlying molecular mechanisms has led to the routine use of the estrogen receptor (ER) as a biomarker of hormone responsiveness, and the development of antiestrogens (AEs) e.g. tamoxifen (TAM), and estrogen deprivation using aromatase inhibitors (AIs) as effective therapies for the treatment and prevention of breast cancer.[3] Indeed, the routine use of adjuvant endocrine therapy is one of the major contributors to the recent decline in breast cancer mortality that has occurred preferentially in ER-positive disease.[2]

Since its first clinical use in the early 1970s, TAM has been the most widely prescribed endocrine treatment for breast cancer in both the advanced disease and adjuvant settings, with a significant impact on survival for patients with endocrine-responsive disease.[2] Although AIs may replace TAM as first-line neoadjuvant and adjuvant endocrine therapy for post-menopausal women in many Westernized countries, TAM will continue to play a critical role in premenopausal women, as a second-line therapy for post-menopausal women, for women in developing countries and in chemoprevention for all breast cancer patients.[4] Indeed, the recent American Society of Clinical Oncology clinical practice guidelines for adjuvant endocrine therapy for ER-positive breast cancer clearly stipulate the use of TAM as a front-line treatment for pre-menopausal women, and either TAM followed by AIs or AIs alone for post-menopausal women.[5]

However, despite its widespread clinical efficacy, the response to TAM is often short-lived, and intrinsic or acquired resistance to endocrine therapy remains a major clinical problem and a significant obstacle to the successful treatment of breast cancer.[6] Furthermore, since disease recurrence decades after diagnosis is not uncommon, it is clear that resistance to TAM will continue to be a significant clinical issue for the foreseeable future. Thus, understanding the molecular basis of AE resistance and developing therapeutic strategies to combat it are major priorities in improving the survival of breast cancer patients, and a significant focus of the global research effort in this area.

In defining the molecular mechanisms underlying the development of endocrine resistance[6] there is emerging evidence of the importance of ligand-independent and non-genomic activation of ER through bi-directional cross-talk with cell surface growth factor receptors and their intracellular signaling cascades.[7] From a clinical perspective, aberrant activation of these growth factor-mediated signaling pathways during malignant progression not only drives breast cancer growth and survival, but is likely to be a major determinant of endocrine response—allowing breast cancer cells to circumvent their dependence upon steroid

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hormones and thus, develop resistance to endocrine therapy.[8,] For example, aberrant epidermal growth factor receptor signaling cascades are independent markers of TAM resistance and decreased survival in clinical breast cancer.[10,11] There is also accumulating evidence of a role for aberrant phosphoinositide-3-kinase (PI3K) signaling—which occurs in the majority of ER-positive breast cancers,[12] in the development of AE resistance.[13,14] Such studies have provided the rationale for examining the efficacy of combining signal transduction inhibitors, in particular those targeting the ERK and PI3K/Akt/mTOR pathways, with endocrine therapy as a strategy for targeting resistant disease.[15,16]

In the current issue of *Cancer Biology & Therapy*,[17] Leung and colleagues used cell models of AE resistance derived from the hormone-responsive breast cancer line, MCF-7 to explore the cellular effects of two dual PI3K/mTOR inhibitors—NVP-BEZ235 (BEZ235)[18] and GSK2126458 (GSK212).[19] The authors had previously developed cellular models of resistance to TAM and AIs by prolonged culturing of MCF-7 cells in increasing concentrations of TAM or in the absence of estrogen, respectively.[20] Interestingly, while the resultant sub-lines displayed some phenotypic heterogeneity, all were resistant to TAM irrespective of how they were derived,[20] suggesting that broad resistance to TAM and AIs may develop via the dysregulation of common, upstream signaling pathways.

The response of these resistant variants to rapamycin—an agent commonly used in the clinic as an immunosuppressant that specifically inhibits a downstream component of PI3K signaling, mammalian target of rapamycin (mTOR), was characterized in earlier work by the same group.[20] The sub-lines demonstrated a differential response to rapamycin treatment, with those derived through estrogen deprivation exhibiting rapamycin resistance which was also associated with a loss of active phospho-HER2 and increased PAX2 expression in these cells. Of interest is the observation that rapamycin induced a marked dephosphorylation of mTOR signaling proteins, such as p70S6k and rpS6 in all of the cell lines, regardless of whether they were sensitive or resistant to its growth inhibitory effects. This led the authors to the somewhat counter intuitive conclusion that activation of the mTOR pathway may not be an effective molecular indicator of rapamycin response.[20]

Leung and colleagues extended this work in their more recent study to determine the effect of AE resistance on the cellular response to the PI3K/mTOR inhibitors, BEZ235 and GSK212.[17] Both of these compounds are highly specific and potent small molecule inhibitors with efficacy against both class I PI3K isoforms and mTOR kinase activity,[18,19] that are currently being evaluated in phase I/II clinical trials either in breast cancer patients with advanced disease (BEZ235), or solid tumors and lymphomas (GSK212). The MCF-7 cell line is an appropriate preclinical model to examine the efficacy of these agents as not only is it an established and well characterized model of ER-positive, luminal breast cancer, but it also harbors a mutation in the helical domain of *PI3KCA*,[21] which encodes the catalytic (p110α) subunit of P13K.

The effects of both inhibitors on cell proliferation, apoptosis, and intracellular signaling through the PI3K/Akt/mTOR pathway were examined in all the resistant sub-lines compared to parental MCF-7 cells. Interestingly, although both inhibitors elicited a significant G1 cell cycle arrest in all of the cell lines studied, this only translated into a decreased growth rate in parental MCF-7s and the TAM resistant variant, TamR7, where proapoptotic effects (determined by measuring the cleavage of PARP) were also observed. This may suggest that, at least in this cell system, the predominant effect of these inhibitors on cell number is mediated via the induction of apoptosis, and certainly there is supporting evidence of a differential, proapoptotic response to BEZ235 in breast cancer cell lines, with evidence that this is associated with HER2 amplification and/or *PIK3CA* mutation, but not PTEN loss of function.[22]

Drawing interesting parallels to their earlier study with rapamycin,[20] Leung et al. observed once again that changes to intracellular signaling components of the PI3K/Akt/mTOR pathway did not correlate with the cellular response to both inhibitors—following treatment, there was a marked hypophosphorylation of p70S6K and rpS6 in all the sub-lines irrespective of their growth response. Furthermore, despite the presence of an activating *PI3KCA* mutation, cells treatment with either inhibitor in combination with TAM, did not reverse the TAM resistance observed in all the sub-lines.

Whilst acknowledging the limitations of any in vitro model, a number of conclusions can be drawn from these data with potential relevance to the clinic. One important point that this study highlights is how the inherent heterogeneity of breast cancers can impinge on the development of resistance and the response to therapy. Although they were derived from the same parental cell line, the resistant variants used in this study displayed marked differences in both their basal phenotype and their response to the PI3K/mTOR inhibitors. Furthermore, the inability of the inhibitors to reverse TAM resistance in these variants differs from the results of Ghayad et al. who demonstrated that the PI3K inhibitor, LY294002 was sufficient to restore TAM sensitivity in their MCF-7-derived model of AE-resistance,[14] highlighting the heterogeneity of experimental models even from the same parental line.

Leung and colleagues also observed an intriguing disconnect between the expression patterns of PI3K/Akt/mTOR signaling components and the actual cellular response to the inhibitors alone and in combination with TAM. This may well reflect the complexities of these intracellular signaling pathways and their regulatory/feedback loops, such that the apparent activation of one signaling cascade does not necessarily predict a tumor's utilization of or dependence on that particular pathway. While this may provide a potential explanation for why such combination therapies for endocrine resistant disease often fall short in the clinic,[15] it does beg the question of 'where now?' for rationale drug design and patient selection for future clinical trials. Clearly, more informative biomarkers of response allowing the better stratification of patients for maximal therapeutic benefit are crucial. Such advances are also dependent upon the continued development and evaluation of

relevant experimental systems that reflect the complexities of breast cancer biology, and delineate the molecular mechanisms driving endocrine resistance in the clinical setting.

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