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Journals Club

Review of: Tamoxifen and TRAIL synergistically induce apoptosis in breast cancer cells

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Abstract of the original article:
Tamoxifen (TAM), is widely used as a single agent in adjuvant treatment of breast cancer. Here, we investigated the effects of TAM in combination with tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) in estrogen receptor-α (ER-α)-positive and -negative breast cancer cells. We showed that cotreatment with TAM and TRAIL synergistically induced apoptosis regardless of ER-α status. By contrast, cotreatment did not affect the viability of normal breast epithelial cells. Cotreatment with TAM and TRAIL in breast cancer cells decreased the levels of antiapoptotic proteins including FLIPs and Bcl-2, and enhanced the levels of pro-apoptotic proteins such as FADD, caspase 8, tBid, Bax and caspase 9. Furthermore, cotreatment-induced apoptosis was efficiently reduced by FADD- or Bid-siRNA, indicating the implication of both extrinsic and intrinsic pathways in synergistic apoptosis induction. Importantly, cotreatment totally arrested tumor growth in an ER-α-negative MDA-MB-231 tumor xenograft model. The abrogation of tumor growth correlated with enhanced apoptosis in tumor tissues. Our findings raise the possibility to use TAM in combination with TRAIL for breast cancers, regardless of ER-α status.

Review

Breast cancer remains a leading cause of female mortality in the Western world. Despite the significant clinical effectiveness of antioestrogen therapies such as tamoxifen (TAM) for patients with oestrogen receptor (ER)-positive disease, therapeutic responsiveness is often short-lived and compromised by the development of resistance [1,2]. Thus, the clinical need for novel therapeutic approaches with increased efficacy has provided the rationale for exploring the interactions between endocrine and non-endocrine therapies as a means of enhancing and broadening responsiveness and overcoming resistance.

As the intricacies of mammalian cell death signalling become further elucidated, the possibility of manipulating the intracellular apoptotic machinery for therapeutic benefit is being explored experimentally [3]. Promising candidates from this approach include BH3 mimetics that act by neutralising pro-survival Bcl-2 proteins [4], and tumour necrosis factor-related apoptosis-inducing ligand (TRAIL). TRAIL is a member

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of the tumour necrosis factor superfamily, and mediates its proapoptotic effects through interaction with cell surface death receptors and the subsequent activation of intracellular cell death cascades [5]. One of the characteristics of TRAIL that marks its potential as an anticancer agent is its apparent specificity for tumour cells without exhibiting toxicity towards normal cells. Although the underlying mechanism for this differential sensitivity remains largely unknown, it may be due to oncoprotein-mediated upregulation of TRAIL receptors [5]. Both recombinant TRAIL and agonistic antibodies targeting TRAIL receptors have shown some early promise in Phase I and II clinical trials [6,7], and the efficacy of TRAIL in combination with other chemotherapeutics is also under investigation.

In this recent study by Lagadec *et al*., the authors examined the previously unexplored interaction between TAM and TRAIL in both ER-positive and ER-negative human breast cancer cell lines. Treatment with either agent alone produced a modest (10–20%) increase in apoptosis and little differential sensitivity between ER-positive and ER-negative cell lines, possibly reflecting the relatively low concentrations of TAM and TRAIL used in this study (2 μM and 1 ng/ml, respectively), and the limits of morphological analysis as a quantifiable method for apoptosis detection. However, results from both Hoescht staining and MTT assays showed that combined treatment with TAM and TRAIL significantly augmented the death response in a synergistic manner.

The investigators went on to perform studies aimed at delineating a possible mechanistic basis for this observed synergy. Flow cytometric analysis of cell cycle distribution revealed that while TAM treatment elicited a G1 arrest in ER-positive, MCF-7 cells, there were no significant, additional effects on cell cycle parameters following combined treatment. However, an increase in the sub-G1 (apoptotic) fraction was observed following single-agent treatment, and this was enhanced in the co-treated cells. These data suggest that either TAM-induced cytostasis is maximal in MCF-7 cells at these concentrations, or more likely, that TRAIL specifically engages TAM-induced apoptotic pathways without enhancing its anti-proliferative effects. Furthermore, the similar proapoptotic effects observed in both ER-positive and ER-negative cells following co-treatment suggest that a significant proportion of this synergistic cytotoxicity is mediated independently of the ER.

TAM-induced apoptosis can proceed via both ER-dependent and ER-independent pathways [8], the latter including the Bcl-2-regulated, intrinsic pathway, and through stress-induced JNK activation. In this study, abrogation of JNK activity using the JNK inhibitor, SP600125 or dominant-negative JNK, reduced the synergistic proapoptotic response suggesting JNK activation may be involved. The investigators also demonstrated evidence of enhanced signalling through both extrinsic and intrinsic apoptotic pathways following co-treatment, such as increased expression of the death receptor adaptor, FADD and the active subunits of caspase-8 and caspase-9, as well as changes in the Bcl-2-like proteins and increased release of mitochondrial cytochrome *c*. Blocking either of these pathways using FADD- or Bid-specific siRNAs did not affect TAM's cytotoxicity, suggesting it is not dependent on the extrinsic pathway or Bid-mediated cross-talk with intrinsic apoptosis. However, siRNA treatment significantly attenuated TRAIL's ability to induce apoptosis, either alone or in combination with TAM. These data demonstrate that enhanced signalling through the extrinsic apoptotic pathway is associated with and required for TRAIL-mediated synergy, and although not specifically addressed by Lagadec and colleagues, it supports previous work showing many chemotherapeutic agents synergise with TRAIL through upregulation of death receptors [9].

The investigators concluded their study with some convincing proof-of-concept studies in an ER-negative breast cancer xenograft model. Tumour volume remained static in mice treated with both TAM and TRAIL, and in support of their *in vitro* work, this appeared to be mediated by an increase in apoptosis rather than changes in the rate of proliferation.

A significant clinical challenge in the treatment of breast cancer remains the lack of responsiveness of tumours to endocrine therapies such as TAM, either due to lack of ER expression or the development of therapeutic resistance. Although preliminary, the experimental work presented in this paper emphasizes the potential of combining TRAIL with TAM to not only enhance the latter's cytotoxicity but to also broaden its efficacy against ER-negative disease. An important focus for further preclinical studies will be to more clearly elucidate the mechanistic basis of TAM and TRAIL-mediated synergy in breast cancer cells, allowing the factors governing responsiveness to be determined. This may include examining the levels of the TRAIL receptors, DR4 and DR5 following co-treatment and extending these studies to a larger panel of breast cancer cell lines, including models of acquired resistance to TAM.

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