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Short Commentary

Invited Commentary Proposing Potential New Treatment Strategies to Combat Breast Cancer Based on our Recently Published Manuscripts

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Most human breast cancers are hormone-dependent; as such they express estrogen and progesterone receptors (ER, PR). Such tumors respond more readily to chemotherapy than tumors that are hormone receptor negative. Women suffering from hormone receptor positive breast cancer are routinely treated with chemotherapeutic drugs such as tamoxifen and aromatase inhibitors, which either block the binding of estrogen to its receptor, or inhibit its synthesis.

Hormone-dependent breast cancer cells frequently express an inactive mutant form of tumor suppressor p53 protein (mtp53). In its wild-type form, p53 promotes apoptosis and cell cycle arrest and inhibits vascular-endothelial growth factor (VEGF)-dependent angiogenesis (formation of new blood vessels), thereby disrupting tumor development. Mtp53 however lacks these functions, resulting in tumor cell survival and metastasis. Conversion of mtp53 to wtp3, with consequent restoration of wild-type tumor suppressor functions occurs when tumors are exposed to the small molecule drug PRIMA-1 (p53 reactivation and induction of massive apoptosis). Using APR-246, a structural analog of PRIMA-1 which likewise restores wtp53 activity, together with an antibody (2aG4) that disrupts tumor vasculature by targeting phosphatidylserine residues on tumor blood vessels, we reduced the viability of BT-474 and T47-D human breast cancer cells, and also suppressed the *in vivo* growth of tumor xenografts in a mouse model of breast cancer [1].

Incubation of BT-474 cells with APR-246 resulted in significantly higher levels of wtp53, reduced VEGF expression and increased expression of genes related to apoptosis. Furthermore, flow cytometry studies showed that APR-246 dose-dependently induced apoptosis and cell death in cultured BT-474 cells. For *in vivo* studies, BT-474 tumor xenografts were grown in nude mice and allowed to develop to a volume of approximately 100 – 125 mm^3 whereupon treatment with APR-246 and/or 2aG4 commenced. Tumor volume was monitored for 40 days. Compared with control animals, mice in all three treatment groups (APR-246, 2aG4 and a combination of the two) exhibited significantly smaller tumors. In animals treated with a combination of APR-246 and 2aG4 tumor volumes were reduced by approximately 65-70% compared with controls, and in some animals tumors were completely eradicated. We observed a similar effect on xenografts derived from T-47D cells, indicating that APR-246 and 2aG4 exert their effects against different human breast cancer cell lines [1]. No signs of toxicity were observed in any of the experimental animals, suggesting that the two agents might be used safely in human subjects afflicted with hormone-dependent breast cancer.

Although the majority of human breast cancers are hormone-dependent, a significant number (approximately 15-20%) are described as triple-negative breast cancer (TNBC). Because they do not express ER and PR, and also lack Her-2-neu, a member of the epidermal growth factor receptor family, TNBCs are distinct from those that are hormone-dependent. By lacking these common chemotherapeutic targets, such cancers are extremely difficult to treat and women afflicted with TNBC often succumb to the disease following metastasis throughout the body. It is therefore imperative that we develop new treatments for this particularly aggressive and deadly form of breast cancer.

A hallmark of TNBC is the presence of mtp53 (in 80% of tumors) and cancer stem-cell like cells which frequently metastasize. While TNBCs are devoid of targetable receptors, expression of mtp53, the inactive form of tumor suppressor, provides a target through which we might treat TNBC. We therefore propose that APR-246 and 2aG4, as well as being effective against hormone-responsive breast cancer, might also be used therapeutically against metastatic TNBC. We will determine whether APR-246 activates mtp53 in TNBC cells and measure its capacity *in vitro* to modulate specific markers of stem cells. We will also carry out studies to ascertain whether migration of TNBC cells is disrupted by APR-246, which would indicate possible suppression of metastasis. Initial observations using an animal model are promising; APR-246 and 2aG4, administered alone or in combination, inhibited metastasis of TNBC and also reduced the number of metastatic colonies formed in the lungs.

During our earlier efforts to determine the mechanism through which PRIMA-1 reduces breast cancer cell viability [2], we serendipitously discovered that inhibition of cholesterol biosynthesis may be an effective means of suppressing breast cancer growth, leading us to investigate another line of research [3]. Based on clinical

trials, there is overwhelming evidence that hormone replacement therapy (HRT) in post-menopausal women increases the risk of breast cancer. HRT regimens may contain estrogen alone, or a combination of estrogen and progesterone, and women undergoing combination therapy are more likely to develop breast cancer than those taking only estrogen.

Using an animal model of progestin-dependent breast cancer, we showed that medroxyprogesterone acetate (MPA), a synthetic progestin widely used in HRT, accelerates development of breast cancer [4]. Studies *in vitro* using cultured T47-D and BT-474 cells showed that MPA elevates levels of CD44 protein, increases ALDH activity and promotes the formation of mammospheres, all hallmarks of cancer stem cells (CSCs) [5]. Because millions of post-menopausal women worldwide take HRT and are therefore susceptible to the potentially deleterious and often fatal effects of progestins, we conducted studies using an inhibitor of cholesterol synthesis, in an attempt to counter such effects.

RO 48-8071 (RO), inhibits 2, 3-oxidosqualene cyclase (OSC), a critical enzyme in the biosynthetic pathway leading to cholesterol production. RO significantly reduced the MPA-induced expression of CD44, lowered levels of PR which were elevated in response to MPA and abolished mammosphere formation [5, 6]. The latter observation suggests that RO interferes with progestin-dependent enrichment of CSCs. By lowering PR levels, RO may affect the growth of tumors in a number of ways; by reducing expression of 1) VEGF, which is extremely angiogenic and promotes breast cancer cell proliferation and tumor development, and 2) CD44, which exists primarily as two variants that play important roles in cell-to-cell communication, cell adhesion and cell migration.

Since cholesterol biosynthesis is essential for cell growth, we will also determine whether RO might be effective against TNBC. Based on our studies to date we are confident that both APR-246 and RO have the potential to be used therapeutically against metastatic breast cancer. Future clinical trials will determine whether these findings can be translated into effective therapies to combat a variety of aggressive breast cancers and thereby alleviate the suffering of millions of women worldwide.

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