Published in final edited form as:
Cell Cycle. 2009 December ; 8(23): 3794–3795.

AGTR1 as a therapeutic target in ER-positive and ERBB2-negative breast cancer cases

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The advent of "personalized medicine" has profoundly altered cancer research and treatment. The goal of personalized medicine for cancer is to identify the specific genetic and/or epigenetic events (which can be therapeutically targeted) that drive an individual patient's breast cancer, rather than assuming that all women with breast cancer have the same disease. Perhaps the best example of this is the identification of amplification and subsequent over-expression of the ERBB2 (Her2) receptor, which can be successfully targeted by the monoclonal antibody trastuzumab, in approximately 25% of patients with breast cancer [1, 2].

Based on this idea, our laboratory previously developed a bioinformatics algorithm (termed COPA) to prioritize genes in DNA microarray data that show marked over-expression in a fraction of samples (such as ERBB2), to identify novel causal cancer genes [3]. Recently, we performed a meta-COPA analysis to query genetic alterations in 31 breast cancer profiling datasets obtained from 3,200 microarray experiments, available in the public domain [3]. This analysis identified ERBB2 and angiotensin II type I receptor (AGTR1) as the top two most consistently high scoring genes, which was intriguing in light of the fact that AGTR1 was previously linked to cancer, and an entire class of AGTR1 receptor blockers (ARBs) are already clinically available [4]. Across all DNA microarray studies AGTR1 was over-expressed in 10–20% of breast cancers, all of which were estrogen receptor (ER) positive and ERBB2-negative. Further, in some tumors, AGTR1 was found to be as much as 100-fold over-expressed [5]. We also showed that ectopic over-expression of AGTR1 in normal breast epithelial cells (with low basal AGTR1 expression), confers an invasive phenotype upon angiotensin II (Ang II) stimulation, which was attenuated by losartan, an FDA approved ARB used clincally to treat hypertension [5]. In light of the fact that AGTR1 always displayed high over-expression in ER-positive and ERBB2-negative tumors from breast cancer samples, this finding provides potential insight into the selective pressures governing AGTR1 activation in breast cancer.

Although we found that a subset of breast cancers with AGTR1 over-expression had copy number gain or amplification of the AGTR1, we did not identify a consistent mechanism for AGTR1 over-expression. Interestingly, estrogen regulatory elements (ERE) and AP-1 have already been identified in the 5′ flanking region of AGTR1 gene, suggesting the possible involvement of estrogen in AGTR1 regulation [6, 7]. Another study showed that estrogen replacement in ovarectimized rats resulted in a decrease in AGTR1 expression in pituitary and

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adrenal glands, whereas there was an increase in such expression in uterus that indicated a more tissue-specific response. The authors also showed that hormonal regulation of AGTR1 expression was mediated by modulation of the 5′ leader sequence of AGTR1 mRNA and cytosolic RNA binding proteins, thus increasing mRNA stability [8]. We hypothesize that estrogen might provide feedback inhibition for the control of estrogen production in the pituitary and adrenal glands, but not in estrogen dependent organs such as the ovaries. Therefore, we plan to explore the role of estrogen in regulation of AGTR1 in breast cancer cells with AGTR1 over-expression. If inhibiting estrogen signaling in breast cancer cell lines decreases AGTR1 expression, then the addition of an ARB to standard anti-estrogen therapy (such as tamoxifen) may be beneficial in women with ER-positive and AGTR1 over-expressing breast cancer.

Thus, the marked over-expression of AGTR1 in a subset of ER positive breast tumors, may be the result of a genetic aberration that placed AGTR1 transcripts under the positive control of the ER. Alternatively, polymorphisms in members of the renin-angiotensin system have been shown to affect Ang II levels and have been linked to an increased risk of breast cancer in some studies [9]. It is unclear if such variations may lead to AGTR1 over-expression. As the exact mechanism of AGTR1 over-expression in breast cancer remains to be elucidated, additional studies that characterize genetic aberrations, sequence variations or mutations, and transcriptional/translational regulation of AGTR1 are needed.

The mutually exclusive expression patterns and reported overlapping downstream pathways affected by AGTR1 and ERBB2 lends support to our hypothesis that the activation of these receptors may represent alternative events in breast tumorigenesis. The ligand for AGTR1, Ang II is known to exhibit cross-talk with several tyrosine kinases via AGTR1, including receptor tyrosine kinases and non-receptor tyrosine kinases [10]. We showed that Ang II stimulation of benign human mammary epithelial (HME) cells that ectopically over-express AGTR1 increases ERK phosphorylation, a MAPK pathway readout [5]. We plan to further investigate the relationship between estrogen and AGTR1 signaling, and examine the possible cellular and molecular mechanisms responsible for the cross-talk between Ang II, AGTR1 and estrogen in the context of breast cancer.

Moreover, Ang II is also known to induce expression of its own precursor, angiotensinogen, via an NF- κB dependent signaling pathways, creating a biological “positive feedback loop” [11]. Signaling pathways leading to Ang II dependent activation of NF- κB involves three principal proteins; CARM3, Bcl10 and MALT1. Blocking the function of any of these proteins effectively abolishes Ang II dependent NF- κB activation in hepatocytes [12]. Altering the CARMA3/Bcl10/MALT1 signaling complex may prevent Ang II dependent NF- κB activation in AGTR1 over-expressing breast cancer cells.

Our *in vivo* studies with control or AGTR1 over-expressing breast cancer xenografts showed differential sensitivity to ARB (losartan) treatment, with AGTR1 over-expressing xenografts demonstrating a 30% decrease in tumor growth at week 8 with losartan treatment, whereas losartan had no effect on control xenografts [5]. We plan to investigate whether other ARBs may have higher efficacies or longer biological half-lives than losartan. Numerous orally active, FDA-approved ARBs have been synthesized and are already available for the treatment of hypertension, such as irbesartan, olmesartan, candesartan, valsartan and telmisartan. Among this class of drugs, telmisartan is structurally unique, possibly rendering it more effective in inhibiting the AGTR1-dependent pro-tumorigenic effects. The structural characteristics not only allows it to effectively block Ang II binding to AGTR1, but also enables it to be a partial agonist for Peroxisome Proliferator-activated Receptor-γ (PPAR-γ), a member of the nuclear receptor family [13].

Another approach for modulating the AGTR1 response could be the development of humanized monoclonal antibody against the extracellular domain of this receptor. A group has shown that a monoclonal antibody (R6315/G2) to a conserved sequence in AGTR1 regressed tumor burden by 74% in MCF7 xenografts [14]. However, by quantitative reverse transcription PCR and in public expression profiling data, we found that MCF7 cells do not over-express AGTR1 (which is the reason we stably over-expressed AGTR1 in MCF7 cells for our xenograft model), and thus it is unclear if inhibiting AGTR1 may also affect breast cancer indirectly through stromal cells or endothelium. However, this antibody has been raised against the region of the receptor that is not involved in hormone binding or signal transduction. It may be more appropriate to explore the different sites of AGTR1 involved in ligand binding and transactivation of other important receptors. Despite the copious amount of data supporting the therapeutic targeting of this receptor, there are currently no targeted humanized monoclonal AGTR1 antibodies in human clinical trials.

In summary, our findings indicate that AGTR1 over-expression identifies a novel subtype of ER-positive, ERBB2-negative breast cancer. We hypothesize that this subset of patients may benefit from targeted therapy with ARBs, such as losartan. AGTR1 antagonists may indeed be a viable therapy for women with AGTR1 over-expressing breast tumors, and this study provides the framework for clinical trials to explore the possible use of AGTR1 antagonists in AGTR1 positive breast cancer patients.

Acknowledgments

We thank Sooryanarayana Varambally for his assistance. This work was supported in part by the National Institutes of Health (to A.M.C.), the Department of Defense and Era of Hope (to A.M.C.) and the Early Detection Research Network (to A.M.C.). A.M.C. is supported by a Burroughs Welcome Foundation Award in Clinical Translational Research, a Doris Duke Charitable Foundation Distinguished Clinical Investigator Award, the Howard Hughes Medical Institute, the American Cancer Society and the Genentech Foundation Postdoctoral Fellowship (to B.A.).

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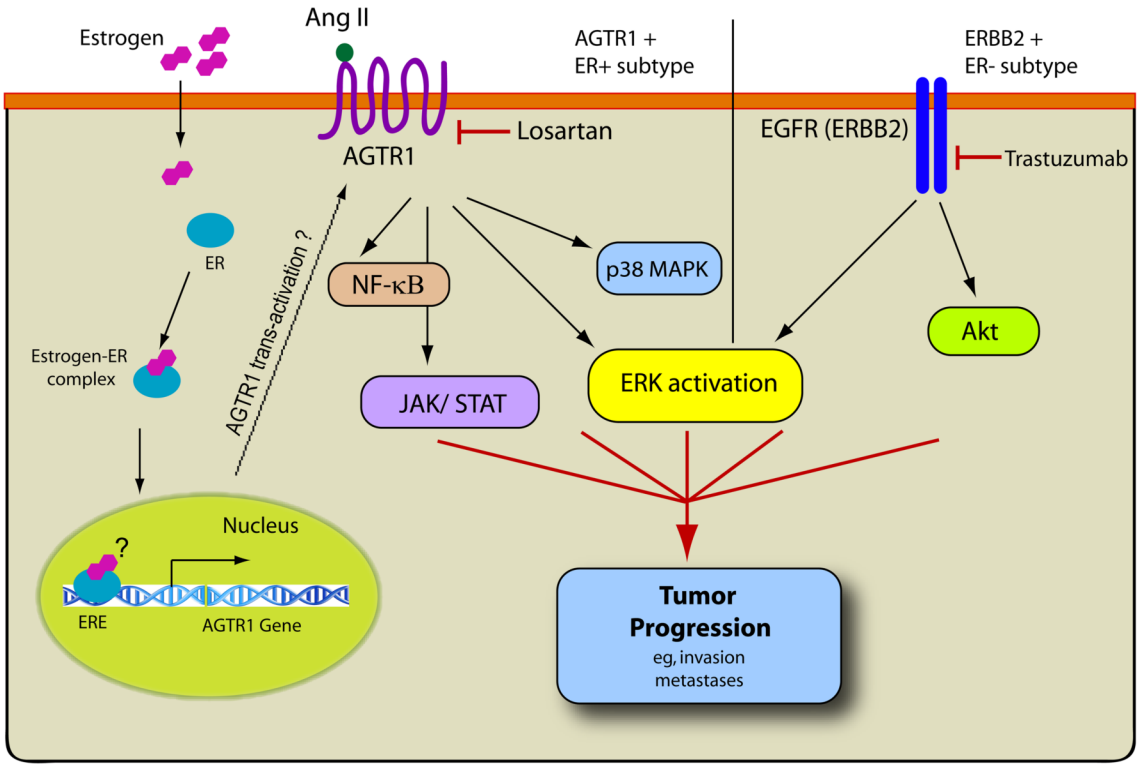

Figure 1.
Mutually exclusive AGTR1+/ER+ and ERBB2+/ER− subtype pathways showing overlap in downstream signaling and a possibility of crosstalk between AGTR1 and estrogen signaling.