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14. ABSTRACT

This project had as its original goal the elucidation of a novel mode of action of the androgen receptor (AR) that may be of importance in the initiation and progression of prostate cancer. Specifically, we had determined that the AR controls the expression of the cell-surface receptor for the hormone IGF-I at the level of translation of the IGF-I receptor mRNA in an androgen-independent fashion. In the course of studies in the first year of funding, we serendipitously found another factor that also regulates IGF-I receptor expression and action, and that also inhibits the expression and action of the EGFR/erbB/HER family of receptor tyrosine kinases. This protein, an alternative product of the HER2 protooncogene termed herstatin, has the potential ability to function as a novel bi-functional inhibitor of both the EGF and IGF signaling systems which are themselves independently implicated in prostate cancer initiation and progression. We propose to re-orient our studies to focus on this potential anti-tumor factor in prostate cancer cells and to determine its potential therapeutic utility. These studies are conceptually in line with our original proposal in that they address a novel mechanism of control of IGF-I receptor expression and action and its role in prostate cancer tumorigenesis and metastasis.

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Introduction

The original purpose of this project was to define a new mechanism of action of the androgen receptor (AR) that may be of relevance to the progression of prostate cancer (CaP). The proposed studies were based upon our previous findings that expression of the AR in AR-negative CaP cells altered the expression of the cell-surface receptor for the growth factor IGF-I. Specifically, AR expression increased the expression of the IGF-I receptor at the translational level in a ligand (i.e., DHT)-independent fashion. The original aims were to determine the regions of the IGF-I receptor gene 5'-UTR and the AR that were necessary for this effect, and to use microarray gene profiling to identify other targets of this novel mode of AR action. In the course of studies in the first year of funding, we serendipitously discovered that another factor also regulates IGF-I receptor expression and action, and also inhibits the expression and action of the EGFR/erbB/HER family of receptor tyrosine kinases. This protein, an alternative product of the HER2 proto-oncogene termed herstatin, has the potential ability to function as a novel bi-functional inhibitor of both the EGF and IGF signaling systems which are themselves independently implicated in prostate cancer initiation and progression. We propose to re-orient our studies to focus on this potential anti-tumor factor in prostate cancer cells and to determine its potential therapeutic utility. These studies are conceptually in line with our original proposal in that they address a novel mechanism of control of IGF-I receptor expression and action and its role in prostate cancer tumorigenesis and metastasis, but are of greater import and translational potential, since they are directed at a druggable target that may also have diagnostic utility.

Body

We have recently discovered that an alternative version of a member of the EGF receptor (EGFR)/HER family of receptor tyrosine kinases (RTKs), termed herstatin, may serve as an endogenous modulator of insulin sensitivity. Herstatin is produced from an alternatively spliced transcript of the HER2/erbB-2 gene that retains intron 8, and is comprised of the amino-terminal 341 amino acids of the HER2 receptor and a unique 79-amino acid carboxyl terminus encoded by the intron 8 sequence (see Figures 1 and 2 below). This secreted molecule, originally cloned from ovarian cancer cells, binds to all members of the EGFF family, including EGFR/HER1/*erb*B1, HER2/*neu*/*erb*B2, HER3/*erb*B3, and HER4/*erb*B4, blocks receptor dimerization and activation, and inhibits signaling by EGF ligands, including EGF, TGF-α, and heregulin [1-5]. Because the IGF-I receptor exhibits partial sequence and structural homology with the EGFR family, we examined the potential interaction of herstatin with the IGF-I receptor. Figure 1 below illustrates the retention of intron 8 that results in the expression of herstatin, which is comprised of the N terminus of HER2 and an intron 8-encoded C terminus that enables the binding of herstatin to the EGFR/HER/erbB family of RTKs (Figure 2).

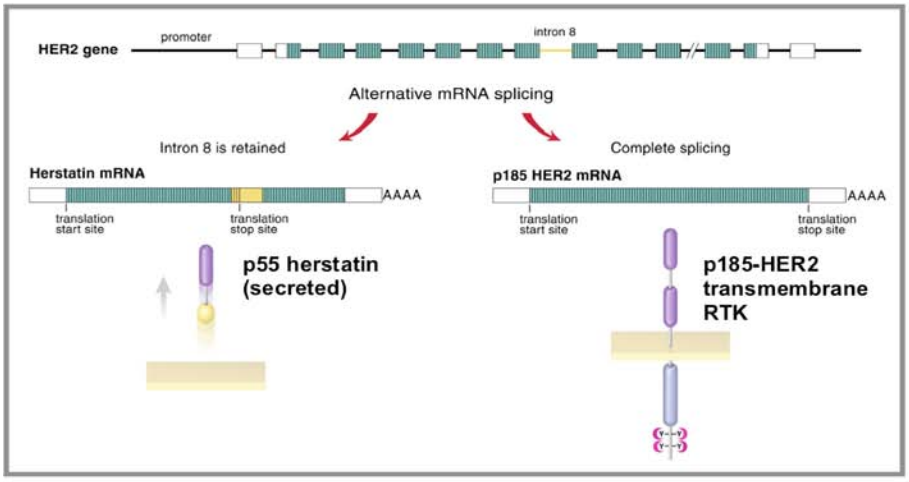

Figure 1. Alternative splicing of intron 8 produces a truncated mRNA encoding herstatin or full-length HER2 mRNA.

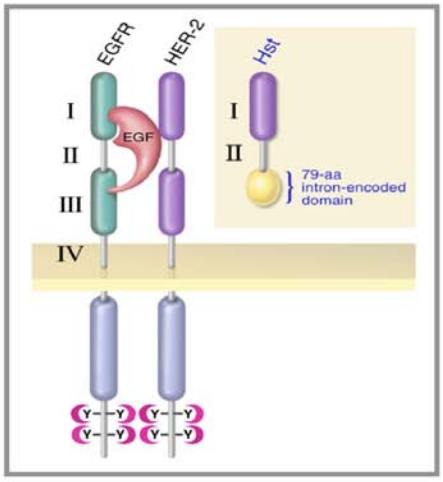

Figure 2. Herstatin domain structure compared to HER2.

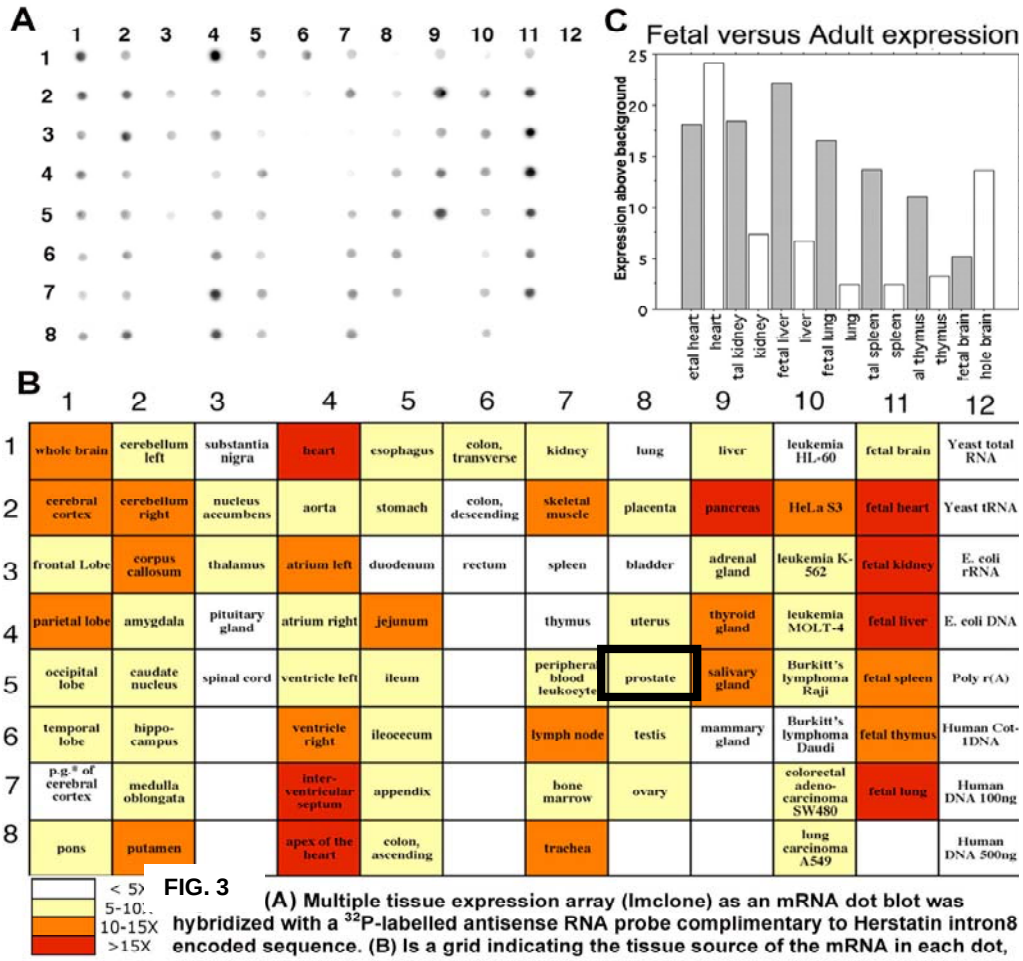

FIG. 3 (A) Multiple tissue expression array (Imclone) as an mRNA dot blot was hybridized with a ^{32}P-labelled antisense RNA probe complimentary to Herstatin intron8 encoded sequence. (B) Is a grid indicating the tissue source of the mRNA in each dot, color coded to indicate the signal above background, quantitated by phosphorImager analysis of the blot. (C) Illustrates the expression in adult versus fetal tissue.

Using an exon 8-specific probe, we have shown that herstatin is expressed in multiple tissues, including prostate (Figure 3A and B; boxed in B), and is developmentally regulated in a number of tissues (Figure 3C).

We initially demonstrated that constitutive expression of herstatin in MCF-7 breast cancer cells effectively inhibited EGF signaling to the ERK and PI3K cascades that are responsible for the proliferative and anti-apoptotic effects of this growth factor. As shown in Figure 4, MCF-7 cells expressing herstatin do not exhibit EGF (14 nM)-stimulated phosphorylation of ERK1/2 (Figure 4A) or Akt/PKB (Figure 4B).

We next addressed the effects of herstatin on IGF-I receptor expression and action. As shown in Figure 5, herstatin-expressing cells contain decreased levels of IGF-I receptor protein and severly diminished IGF-I-stimulated IGF-I receptor activation as assessed by receptor autophosphorylation. In parallel, the levels and activation of the IRS-1 scaffolding/adaptor protein that links the IGF-I receptor to downstream signaling cascades was also reduced in herstatin-expressing cells. Interestingly, however, the levels and activation of IRS-2 were not affected by herstatin expression (Figure 6).

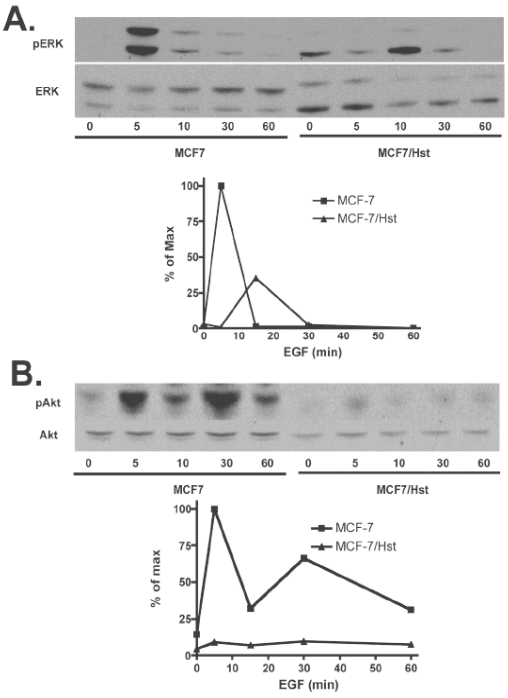

Figure 4. Inhibition of EGF-stimulated ERK (A) and Akt/PKB (B) activation in herstatin-expressing MCF-7 cells.

In additional experiments, we have shown that, as was the case with EGF-stimulated signaling, herstatin-expressing cells were resistant to IGF-I activation of the ERK and PI3K pathways that are responsible for the proliferative and anti-apoptotic effects of IGF-I (data not shown). As a result of this inhibition of IGF signaling, herstatin inhibited the ability of IGF-I to stimulate cellular proliferation in two independent herstatin-expressing MCF-7 clones (Figure 7).

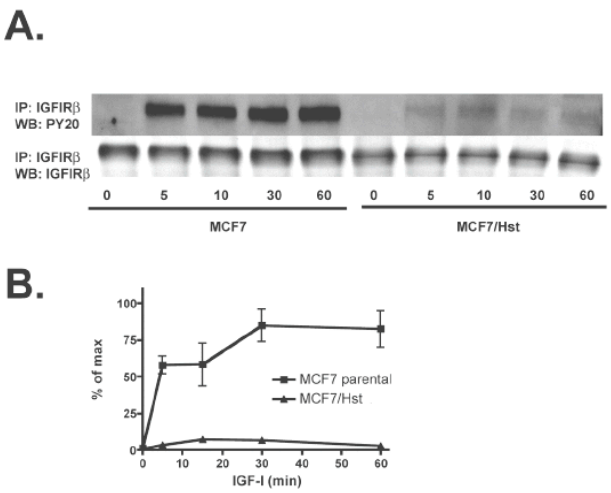

Figure 5. Inhibition of IGF-I receptor expression and activation in herstatin-expressing MCF-7 cells.

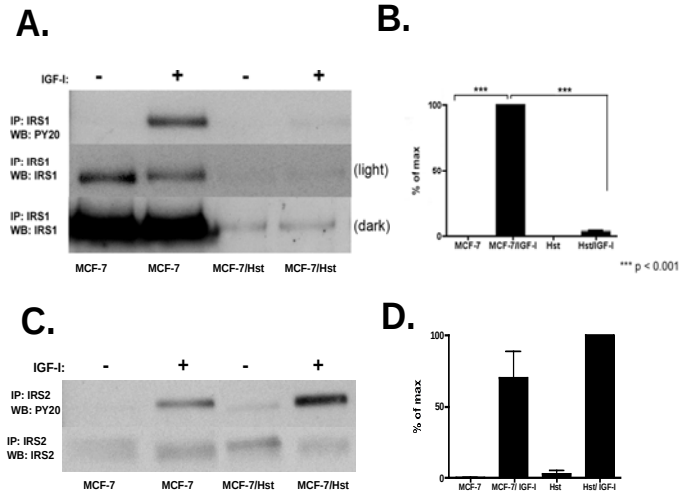

Figure 6. Inhibition of IRS-1 (A,B), but not IRS-2 (C,D) expression and activation in herstatin-expressing MCF-7 cells.

Although herstatin binds to the IGF-I receptor and down-regulates IGF-I signaling, it is possible that the observed effects of herstatin on IGF-I action are mediated through its inhibition of EGF signaling per se. To address this possibility, we evaluated the ability of an EGFR inhibitor, AD1478, to mimic the effects of herstatin on IGF-I receptor expression and action. As shown in Figure 8, AG1478, at a concentration that effectively inhibits EGFR signaling, did not replicate the effects of herstatin on inhibition of IGF-I receptor expression. Thus, the effects of herstatin depicted in the data above appear to be a direct result of modulation of IGF-I receptor expression and action.

These studies provide the foundation for our proposed re-orientation of our aims to exploit the potential of herstatin as a bifunctional inhibitor of EGFR and IGF system expression and function in the context of prostate cancer.

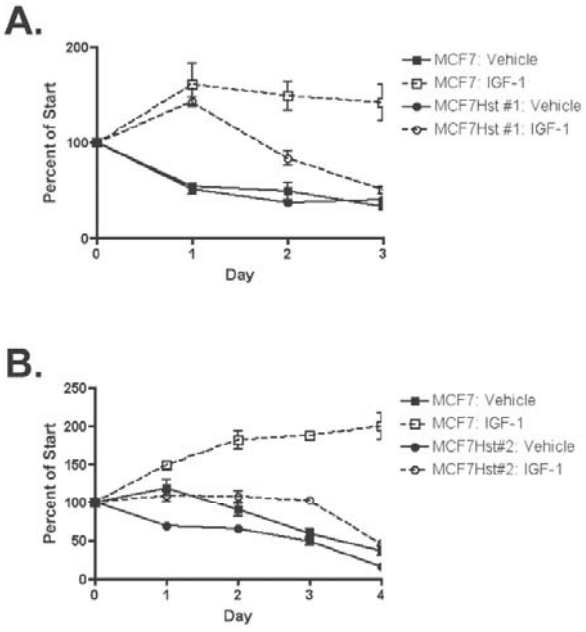

Figure 8. Lack of an effect of an EGFR inhibitor on IGF-I receptor expression.

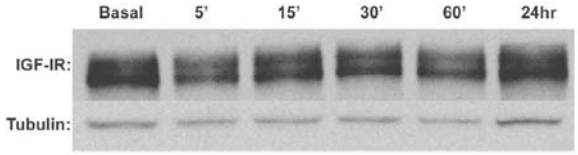

Figure 7. Inhibition of IGF-I-stimulated growth in herstatin-expressing MCF-7 clones.

Key research accomplishments

- Demonstration of herstatin interaction with IGF-I receptor at nM concentrations

- Characterization of tissue-specific expression, including normal prostate tissue
- Description of inhibitory effects of herstatin on IGF receptor expression and activation
- Characterization of altered downstream signaling by herstatin
- Demonstration of growth-inhibitory effects of herstatin
- Preliminary mapping of herstatin transcriptional unit

Reportable outcomes

None (2 manuscripts in preparation).

Conclusions

The studies above demonstrate the potential role of herstatin in modulating the expression and action of both the EGF and IGF signaling systems in prostate cancer. Additionally, our findings suggest that herstatin may be a tractable therapeutic target in prostate cancer. Specifically, it will be important to assess the effect of herstatin on prostate cancer cell behavior, the differential expression of herstatin in normal and cancerous prostate tissue, and the mechanisms that regulate endogenous herstatin expression. Toward this end, we request an alteration of the original statement of work to reflect our focus on these exciting new data.

Proposed revised Statement of Work

Task 1. Characterize the effects of herstatin in prostate cancer cells (months 7-12 of year 2 and 1-6 of year 3).

a. *Generate lentivirus constructs encoding herstatin and infect androgen-sensitive (LNCaP) and insensitive (PC-3) CaP cell lines.*
b. *Analyze IGF-I signaling in acutely and long-term infected cells.*
c. *Assess effects of herstatin on proliferation, migration, and apoptosis.*

Task 2. Evaluate expression of herstatin in prostate cancer cell lines and clinical samples (months 1-12 of year 3).

a. *Optimize quantitative RT-PCR amplicon probesets and determine herstatin vs HER2 mRNA levels in a series of CaP cell lines, including PC-3, PC-3/AR, LNCaP, DU-145, P69, M12, and 22rV.1.*
b. *Assay herstatin expression in normal prostate tissue and CaP biopsies (already obtained from OHSU Cancer Center tissue bank).*

Task 3. Investigate regulation of herstatin expression (months 7-12 of year 2 and 1-12 of year 3).

a. *Based upon preliminary data suggesting that heregulin increases herstatin expression in SKOV-3 cells, we will determine the effects of EGF system ligands on herstatin mRNA levels in CaP cell lines that express endogenous herstatin.*
b. *Utilizing minigene constructs that allow the assessment of intron 8 retention, evaluate differential intron retention activity in CaP cell lines and the effect of EGF ligands on minigene activity.*
c. *Determine the effect of WT1 (+KTS) on herstatin expression and minigene activity. This experiment is based upon a recent report that a particular splice variant of the WT1 tumor suppressor (which we have previously shown to be present in CaP [6] regulates intron retention and the translation of mRNAs containing retained introns [7].*

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Appendices

None.

Supporting data.

None.