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Review of: Oncogenic transformation of human mammary epithelial cells by autocrine human growth hormone

C. J. Ormandy

Garvan Institute of Medical Research, Darlinghurst, NSW, Australia.

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Abstract of the original article

The human growth hormone (hGH) gene is expressed in the normal human mammary epithelial cell and its expression increases concomitantly with the acquisition of proliferative lesions. Herein we demonstrate that autocrine production of hGH in human mammary carcinoma cells dramatically enhances anchorage-independent growth in a Janus kinase 2-dependent manner. Forced expression of the hGH gene in immortalized human mammary epithelial cells increased proliferation, decreased apoptosis, altered the cellular morphology and resulted in oncogenic transformation. Autocrine hGH was therefore sufficient to support anchorage-independent growth of immortalized human mammary epithelial cells and tumor formation *in vivo*. Moreover, autocrine hGH disrupted normal mammary acinar architecture with luminal filling and deregulated proliferation in three-dimensional epithelial cell culture. Autocrine hGH utilized homeobox A1 to govern the transcriptional program required for autocrine hGH-stimulated oncogenic transformation of human mammary epithelial cells, including transcriptional up-regulation of c-Myc, cyclin D1, and bcl-2. Forced expression of a single orthotopically expressed wild-type gene is therefore sufficient for oncogenic transformation of the immortalized human mammary epithelial cell.

Review

Growth hormone and prolactin are pituitary hormones that circulate to influence a large number of physiological processes. One target of these hormones is the mammary gland where they regulate normal development via their endocrine action. Growth hormone receptors are located throughout the mammary gland. Ductal development fails during puberty in growth hormone receptor knockout mice, but proceeds to a level sufficient for lactation during pregnancy [1]. Prolactin receptors are predominantly located on the mammary epithelial cells, and consequently glands formed from prolactin receptor null epithelium fail to develop lobuloalveolar during pregnancy [2]. Both of these hormones can also be produced by the mammary gland under normal physiological conditions, suggesting that they may have an additional autocrine role. Forced over expression of human growth hormone (hGH) or prolactin [3] in the mouse mammary gland results in mammary cancer via activation of the prolactin receptor [4], as hGH also activates the prolactin receptor. Loss of the prolactin gene from the epithelium only has no effect on development, but results in reduced cell

Correspondence to: Christopher J. Ormandy, Associate Professor, Garvan Institute of Medical Research, Darlinghurst, NSW 2010, Australia. E-mail: c.ormandy@garvan.org.au; Tel: +61 2 9295 8329; Fax: +61 2 9295 8321

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proliferation in late pregnancy, indicating a biological role for physiological production of autocrine prolactin [5]. The prospective Nurse's Health Study has shown that high serum prolactin is associated with elevated relative risk of breast cancer [6,7]. In contrast, an effect of endocrine growth hormone has not been demonstrated in mammary or breast cancer [8].

Against this background, Peter Lobie and colleagues have explored the consequences of over expression of hGH (autocrine hGH) in cells of mammary origin. Initial experiments characterized pooled stable transfectants of human breast cancer cells expressing hGH, MCF-7–hGH. The parent (MCF-7) and control (MCF-7–MUT) cell lines showed an increase in cell number in response to treatment with exogenous hGH, while the MCF-7–hGH cells showed an increase in cell number in the absence of hGH. This increase was muted in response to exogenous hGH and abrogated by the GH antagonist G120R or inhibitors of the Map kinase pathway [9]. Later studies showed that the hGH antagonist B2036 (which unlike G120R [10] does not interact with the prolactin receptor [11]) can also abrogate the effects of autocrine hGH [12]. A publication in 2003 provided the first hints that oncogenesis could result from autocrine hGH but also that the mechanism behind these observations was not as simple as presumed. Autocrine hGH increased expression and activity of the HOXA1 oncogene, but 'although exogenous hGH increased HOXA1 mRNA, it did not result in an increase in HOXA1 protein nor transcriptional activity' [13]. Work published later that year showed that autocrine hGH produced an epithelial to mesenchymal transition (EMT) in MCF-7 cells and conferred migratory and invasive phenotypes, both as cell cultures and when grown as xenografts in mice [14].

The oncogenic consequences of autocrine hGH along with contrasting actions of exogenous and autocrine hGH were further explored in the paper that is the subject of this review [15]. MCF-7–hGH cells showed colony formation in soft agar that was not reproduced by exogenous treatment with hGH of the control MCF-7–MUT cells. When autocrine hGH was expressed in non-tumorigenic MCF-10A cells the same result was obtained. The MCF-10A-hGH cells showed faster cell proliferation, resistance to apoptosis, and a stellate growth pattern on matrigel. When cultured in three dimensions the MCF-10A–hGH cells proliferated more than the parent cell line and did not undergo luminal apoptosis. As xenografts they were tumorigenic in contrast to the controls, where no tumors occurred during the time of the experiment. Autocrine hGH also transformed NIH-3T3 cells. Returning to mechanism, HoxA1 regulation by autocrine hGH increased c-myc, cyclin D1, bcl-2, and hTERT expression, the later the subject of a future publication. Thus autocrine expression of hGH results in mammary carcinogenesis.

A key point to be established is whether autocrine expression of hGH is seen in human breast cancer and whether it contributes to pathogenesis. Does autocrine hGH expression correlate with measurable disease parameters such as tumor size, grade and metastasis, or does it confer a change in the rate of tumor progression and subsequent patient outcome? It may be that autocrine hGH is a factor in the generation of early proliferative lesions that is lost in advanced cancer. Thus, any further study aimed at addressing these issues should include normal breast, early proliferative lesions, and ductal carcinomas *in situ*. It is also likely that this event would be coupled to the generation of histological changes in the stromal compartment, as xenografts of MCF-7–hGH cells produce a strong stromal reaction [14].

The failure of exogenous hGH to reproduce many of the effects of autocrine hGH is intriguing. Interestingly, the same observation has been made for autocrine prolactin [16]. A number of hypotheses have been proposed. Lobie and colleagues speculate that it maybe the continual exposure to growth hormone produced by vector based over expression that is not reproduced by the addition of GH to the media [15]. A pulsatile pattern is seen in pituitary secretion in humans, which when reproduced *in vitro* produced changes in Stat5b activation that are distinct from continual exposure [17]. A simple test of this hypothesis would be a co-culture experiment where hGH expressing cells provide a constant supply of hGH to normal cells. Alternatively it may be that autocrine hGH is not acting entirely at the cell surface, but instead at the Golgi or endoplasmic reticulum in a way that escapes negative regulation by the SOCS protein, or in the nucleus via a novel mechanism [8]. It is possible that autocrine hGH elicits the production of other growth factors by the cell, such as the insulin growth factors (IGFs). A key aspect of these hypotheses is that vector driven over expression may have disrupted the cell's normal sub-cellular partitioning of hGH. Perhaps intracrine hGH may prove to be a more correct description of hGH action during forced expression.

Another issue is the role of the prolactin receptor signaling pathway. The effect of autocrine hGH is clearly dependent on the presence of the growth hormone receptor, as demonstrated by the ability of growth hormone antagonists to prevent a number of actions of autocrine hGH [12]. This does not exclude the potential for hGH activation of the prolactin receptor to contribute to the effect of autocrine hGH. It also remains possible that the GH antagonists used possess undiscovered and species specific

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actions via the prolactin receptor [10]. It would be interesting to know if antagonists of the prolactin receptor (human prolactin G129R or S179D), or knock down of the prolactin receptor can modulate the action of autocrine hGH, to conclusively include or exclude this mechanism. It is possible that autocrine expression of hGH in some way allows hGH to inappropriately activate both the growth hormone and prolactin receptor, stimulating two pathways to provide the proliferative, antiapoptotic, EMT, and invasive characteristics required for carcinogenesis.

References

1. Gallego MI, Binart N, Robinson GW, *et al*. Prolactin, growth hormone, and epidermal growth factor activate Stat5 in different compartments of mammary tissue and exert different and overlapping developmental effects. *Devel Biol* 2001; **229**: 163–175.
2. Harris J, Stanford PM, Oakes SR, Ormandy CJ. Prolactin and the prolactin receptor: new targets of an old hormone. *Ann Med* 2004; **36**: 414–425.
3. Rose-Hellekant TA, Arendt LM, Schroeder MD, *et al*. Prolactin induces ERalpha-positive and ERalpha-negative mammary cancer in transgenic mice. *Oncogene* 2003; **22**: 4664–4674.
4. Wennbo H, Tornell J. The role of prolactin and growth hormone in breast cancer. *Oncogene* 2000; **19**: 1072–1076.
5. Naylor MJ, Lockefeer JA, Horseman ND, Ormandy CJ. Prolactin regulates mammary epithelial cell proliferation via autocrine/paracrine mechanism. *Endocrine* 2003; **20**: 111–114.
6. Hankinson SE, Willett WC, Michaud DS, *et al*. Plasma prolactin levels and subsequent risk of breast cancer in postmenopausal women. *J Natl Cancer Inst* 1999; **91**: 629–634.
7. Tworoger SS, Eliassen AH, Rosner B, *et al*. Plasma prolactin concentrations and risk of postmenopausal breast cancer. *Cancer Res* 2004; **64**: 6814–6819.
8. Waters MJ, Conway-Campbell BL. The oncogenic potential of autocrine human growth hormone in breast cancer. *Proc Natl Acad Sci USA* 2004; **101**: 14992–14993.
9. Kaulsay KK, Mertani HC, Tornell J, *et al*. Autocrine stimulation of human mammary carcinoma cell proliferation by human growth hormone. *Exp Cell Res* 1999; **250**: 35–50.
10. Mode A, Tollet P, Wells T, *et al*. The human growth hormone (hGH) antagonist G120RhGH does not antagonise GH in the rat, but has paradoxical agonist activity, probably via the prolactin receptor. *Endocrinology* 1996; **137**: 447–454.
11. Goffin V, Bernichtein S, Carriere O, *et al*. The human growth hormone antagonist B2036 does not interact with the prolactin receptor. *Endocrinology* 1999; **140**: 3853–3856.
12. Kaulsay KK, Zhu T, Bennett W, *et al*. The effects of autocrine human growth hormone (hGH) on human mammary carcinoma cell behavior are mediated via the hGH receptor. *Endocrinology* 2001; **142**: 767–777.
13. Zhang X, Zhu T, Chen Y, *et al*. Human growth hormone-regulated HOXA1 is a human mammary epithelial oncogene. *J Biol Chem* 2003; **278**: 7580–7590.
14. Mukhina S, Mertani HC, Guo K, *et al*. Phenotypic conversion of human mammary carcinoma cells by autocrine human growth hormone. *Proc Natl Acad Sci USA* 2004; **101**: 15166–15171.
15. Zhu T, Starling-Emerald B, Zhang X, *et al*. Oncogenic transformation of human mammary epithelial cells by autocrine human growth hormone. *Cancer Res* 2005; **65**: 317–324.
16. Gutzman JH, Miller KK, Schuler LA. Endogenous human prolactin and not exogenous human prolactin induces estrogen receptor alpha and prolactin receptor expression and increases estrogen responsiveness in breast cancer cells. *J Steroid Biochem Mol Biol* 2004; **88**: 69–77.
17. Gebert CA, Park SH, Waxman DJ. Down-regulation of liver JAK2-STAT5b signaling by the female plasma pattern of continuous growth hormone stimulation. *Mol Endocrinol* 1999; **13**: 213–227.