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Review of: Bone marrow stromal cell-derived growth inhibitor inhibits growth and migration of breast cancer cells via induction of cell cycle arrest and apoptosis

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Abstract of the original article
Genes encoding growth-inhibitory proteins are postulated to be candidate tumor suppressors. The identification of such proteins may benefit the early diagnosis and therapy of tumors. Here we report the cloning and functional characterization of a novel human bone marrow stromal cell (BMSC)-derived growth inhibitor (BDGI) by large scale random sequencing of a human BMSC cDNA library. Human BDGI cDNA encodes a 477-amino acid residue protein that shares high homology with rat and mouse pregnancy-induced growth inhibitors. The C-terminal of BDGI is identical to a novel human pregnancy-induced growth inhibitor, OKL38. BDGI is also closely related to many other eukaryotic proteins, which together form a novel and highly conserved family of BDGI-like proteins. BDGI overexpression inhibits the proliferation, decreases anchorage-dependent growth, and reduces migration of MCF-7 human breast cancer cells, whereas down-regulation of BDGI expression promotes the proliferation of MCF-7 and HeLa cervix epitheloid carcinoma cells. Interestingly, the inhibitory effect of BDGI on MCF-7 cells is more potent than that of OKL38. We demonstrate that BDGI induces cell cycle arrest in S phase and subsequent apoptosis of MCF-7 cells, which is likely to account for the antiproliferative effects of BDGI. This process may involve up-regulation of p27Kip1 and down-regulation of cyclin A, Bcl-2, and Bcl-xL. The inhibitory effect of BDGI on cell proliferation and the induction of apoptosis were also observed in A549 lung cancer cells but not HeLa cells. These results indicate that BDGI might be a growth inhibitor for human tumor cells, especially breast cancer cells, possibly contributing to the development of new therapeutic strategies for breast cancer.

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Review

Breast cancer remains a leading cause of mortality in the Western world. Although the factors leading to the development of this disease remain largely undefined, there is evidence to suggest that endogenous

growth factors and inhibitors play an important role in regulating normal and malignant growth in the breast epithelium [1]. Signalling cascades downstream of epidermal growth factor, insulin-like growth factors, and transforming growth factor-α and -β have all been shown to influence proliferation in normal and malignant breast epithelial cells, leading to the possibility of such pathways being manipulated for therapeutic benefit [2]. There have also been reports of mammary-specific growth regulators, such as the mammary-derived growth inhibitor (MDGI) [3] and the related protein, MDGI-related gene, MRG [4]. Both these proteins are expressed in the normal mammary epithelium but not in breast carcinomas [4,5], and both have potent, inhibitory effects on the growth of human breast cancer cells *in vitro* [3–5] and in nude mouse models [3,4], supporting their role as candidate tumour suppressors for breast cancer. Interestingly, there is evidence that MDGI and MRG regulate the differentiating effects of pregnancy, suggesting they may mediate the protective effects of early parity against the development of breast cancer.

Huynh and colleagues recently identified and characterized a novel growth inhibitor of mammary epithelial cells, OKL38 [6,7], that is localised to breast epithelial cells and induced during pregnancy and lactation. OKL38 expression is low in breast cancer cell lines and in dimethylbenz(a)anthracene-induced breast tumours, and stable overexpression of OKL38 has inhibitory effects on the growth of MCF-7 human breast cancer cells, both *in vitro* and *in vivo* [6]. Further evidence of its role as a potential tumour suppressor has been demonstrated in later studies by the same group, where they showed that OKL38 mRNA is down-regulated in 70% of kidney tumours and OKL38 protein is undetectable in 78% of kidney tumour tissue compared to paired normal controls [7].

This recent study by Wang *et al.* [8] describes a novel growth-inhibitory factor for breast cancer cells, designated bone marrow stromal cell (BMSC)-derived growth inhibitor (BDGI). BDGI was isolated from a cDNA library of human BMSCs by large-scale random sequencing, and is a 477-residue protein with predicted molecular mass of 51.99 kDa. The lack of both an N-terminal signal peptide and transmembrane regions, suggests that BDGI may be a nonsecretory, soluble protein. Interestingly, BDGI is identical to OKL38 at the C-terminus, although it is 160 residues longer than OKL38 at the N-terminus. Both also share an identical location on chromosome 16q23.3, leading the authors to conclude that BDGI is a longer variant of OKL38. Further homology analysis demonstrated a significant association between BDGI and pregnancy-induced growth inhibitors in rat and mouse. These data led the authors to hypothesise that BDGI may also act as negative regulator of breast epithelial cell growth.

The authors examined BDGI mRNA levels in several human tissues, although, surprisingly not in breast tissue, and high expression in testis, liver and skeletal muscle was demonstrated. Interestingly, this expression pattern is similar to that seen with OKL38, where high expression in liver and testis was also observed [7]. BDGI protein and mRNA analysis of several tumour cell lines also showed differential expression, with relatively low mRNA and protein levels in the human breast cancer cell line, MCF-7. Overexpression of BDGI in these cells inhibited *in vitro* markers of proliferation and transformation to a greater extent than that observed with overexpression of OKL38, although comparable expression levels of the two proteins were not shown. Despite its low endogenous expression, knockdown of BDGI expression with siRNA accelerated MCF-7 cell proliferation.

To determine a possible mechanism for these antiproliferative actions, the authors examined the effects of BDGI on cell cycle progression and apoptosis using transiently transfected MCF-7 cells. BDGI expression led to a significant accumulation of cells in S phase which, together with the decrease in [^{3}H] thymidine incorporation, led the investigators to conclude that these cells were arrested in S phase. This arrest appeared to be associated with a transient decrease in cyclin A expression and increased expression of the cyclin-dependent kinase inhibitor, $p27^{Kip1}$. The investigators also showed a significant increase in apoptosis in BDGI-expressing cells compared to controls, with some evidence that this may occur *via* a modulation of Bcl-2 family proteins.

In the quest for improved therapeutics and diagnostics for breast cancer, there is clearly a need for greater understanding of the factors influencing normal and malignant breast cell growth. Several studies have described endogenous growth inhibitors that have specific antiproliferative effects on breast cancer cells, and may have some potential as antitumour agents [9]. The interesting preliminary characterisation of BDGI presented in this paper, suggests that it may be an important regulator of breast cancer cell growth. However, complex regulatory mechanisms govern both normal and malignant breast epithelial cell growth, and further mechanistic studies are required to elucidate a growth-regulatory role for BDGI in the human breast. For example, establishing expression levels in normal and malignant breast tissue, identifying upstream regulators and determining the effects of BDGI in *in vivo* breast tumour models would be critical steps to further establish the potential efficacy of this factor as a breast cancer therapeutic agent.

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