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The Glycoprotein Growth Factor Progranulin Promotes Carcinogenesis and has Potential Value in Anti-cancer Therapy

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Abstract

Progranulin (PGRN) is a secreted glycoprotein growth factor with tumorigenic roles in a variety of tumors including, among others, breast, ovarian, prostate, bladder, and liver cancer. In some patients, for example with breast, ovarian or liver cancers, high PGRN expression in tumors correlated with a worse outcome. Studies using cell lines and animal models provide evidence that PGRN promotes tumor cell proliferation, migration and survival, and induces drug resistance. Increasing or decreasing PGRN production enhances or inhibits respectively the growth of PGRN-sensitive tumors *in vivo*. PGRN activity is associated with p44/42 mitogen-activated protein kinase as well as phosphatidylinositol 3-kinases signaling pathways. In addition, PGRN may stimulate the formation of the tumor stroma. As an extracellular regulator of tumorgenesis, PGRN is a potential therapeutic target and biomarker of prognosis in the treatment of various cancers.

Introduction

Growth factors, their receptors and downstream signaling proteins play a cardinal role in carcinogenesis [1]. In addition, many cancers depend upon hormones such as estrogen or androgens to support their growth. The importance of these extracellular signaling systems as targets in the development of anti-cancer drugs has long been recognized. Despite this progress, cancer continues to be one of the major causes of morbidity and mortality and many types of cancer remain plagued by a high loss of life [2]. The search for additional biological targets to expand the arsenal of potential anti-cancer weapons remains a priority. Here we will explore the hypothesis that progranulin (PGRN) is an extracellular regulator of tumor progression that presents novel opportunities as a therapeutic and prognostic target in the treatment of a variety of different cancers. The evidence will be reviewed (i): that PGRN is often over-produced in cancerous tissue and that this over-production correlates with disease progression, (ii): that experimentally increasing or decreasing the production or bioavailability of PGRN by cancer cells makes them respectively more or less tumorigenic *in vivo*, (iii): that PGRN stimulates mitosis, blocks apoptosis, including apoptosis due to anti-cancer drugs, and promotes invasion and that this occurs through signaling pathways that are well known to have oncogenic potential, (iv): that PGRN may have additional tumor promoting roles, for example by eliciting tumor stroma production.

Progranulin [3] is also known as Granulin-Epithelin Precursor [4], Proepithelin [5], PC-cell-derived growth factor (PCDGF) [6], Acrogranin [7] and Glycoprotein, 88kDa (GP88). Edman sequencing of a protein called epithelial transforming growth factor (TGFe) revealed a sequence almost identical to an internal portion of PGRN, suggesting that TGFe may be a biologically active fragment of PGRN [8]. PGRN is a secretable glycoprotein [9] consisting of tandem repeats of a 12 cysteine module called the granulin or epithelin domain [10-12,5,7]. The individual granulin/epithelin modules can be isolated from tissue extracts and urine as individual peptides of approximately 6 kDa [10,11,13], but whether these are biologically active in their own right or simple breakdown products is not fully resolved (See Figure 1). In this article the term PGRN (progranulin) will be used to mean the full length protein and is synonymous with Granulin/Epithelin Precursor, Proepithelin, PC-Cell-derived growth factor, acrogranin and epithelial Transforming Growth Factor. The 6 kDa peptidic fragments that correspond to individual granulin/epithelin modules will be called grn/epi peptides. Following NCBI usage the gene will be referred to as *GRN*.

PGRN is a multifunctional protein that has been implicated in early embryonic development [14,15], bone development [16], inflammation [17-19] and wound repair [20]. Mutational loss of a single copy of the human *GRN* gene results in a form of early onset dementia, called frontotemporal lobar dementia that is characterized by neuronal atrophy in the frontal and temporal cerebral lobes [21,22]. Many of these proposed biological roles for PGRN are characterized by cell proliferation, improved survival in the face of an apoptotic challenge, and migration through an extracellular matrix. As will be discussed below it is through these activities that PGRN is thought to promote cancer growth and development.

PGRN expression in tumors from different anatomical sites

PGRN is expressed at higher levels than normal in a number of cancers of different types including carcinomas, sarcomas, gliomas and myelomas. The diversity of anatomical sites in which PGRN has been associated with cancer is suggestive of a significant role for PGRN in tumor biology. Although hard and fast rules cannot be applied, there is a trend, as reviewed below, for PGRN production to be greatest in more invasive stages of cancer progression. In some cases PGRN levels correlate well with clinical parameters such as overall survival and

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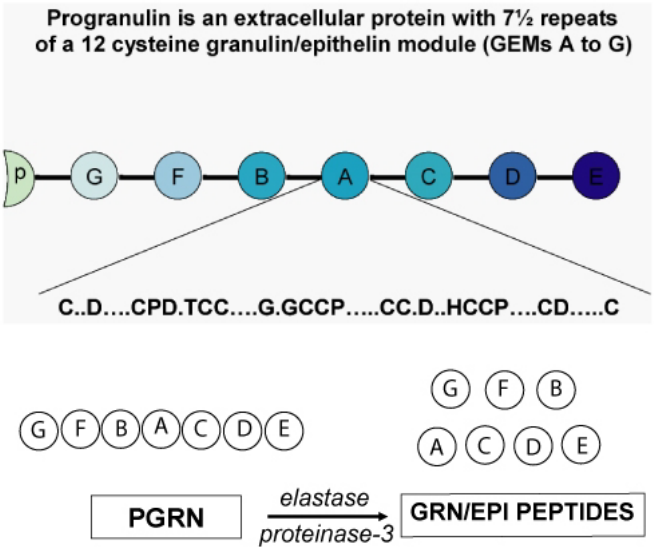

Figure 1: The structure of Progranulin: The upper panel shows that progranulin is a glycoprotein that is composed of seven and a half repeats of the granulin/epithelin module designated A to G. P represents paragranulin, a six cysteine half module. The conserved amino acids of the granulin/epithelin module are shown (where C is cysteine, G is glycine, T is threonine, D is aspartic acid, P is proline and H is histidine. Dots represent amino acid residues that are not highly conserved). The lower panel shows that progranulin can be converted to peptide fragments each of about 6kDa that correspond to the granulin/epithelin modules.

progression free survival.

Breast cancer: Immunohistochemical analysis of paraffin-embedded human breast cancer samples demonstrated that PGRN was overexpressed in 80% [23] and 79% [24] of invasive ductal carcinoma and half of invasive lobular carcinoma, whereas almost no detectable PGRN expression was observed in normal mammary epithelium and benign tumors. PGRN expression significantly correlated with the histological grade of invasive and ductal carcinomas in situ [23], and with the Ki-67 index of proliferation in all invasive carcinomas [23]. P53 was more commonly expressed in those invasive carcinomas with high PGRN expression [23,24]. There was no significant correlation between PGRN expression and the human epidermal growth factor receptor (HER) family member HER-2. PGRN expression was independent of estrogen and progesterone receptors (ER/PR) status in one study [23], whereas Song et al. [24] reported that PGRN positive staining was more common in ER/PR negative samples than that in ER/PR positive tumors. Elkabets et al. [25] reported that high level PGRN staining was significantly associated with breast tumor size, histological and molecular subtypes. High PGRN expression correlated negatively with the luminal A subtype which are generally low grade tumors that express ER [26], but was positively correlated with triple negative breast cancer (tumors with negative expression of ER, PR, and HER-2 [27]) and basal-like breast cancer subtypes [25]. These cancers are often difficult to treat, suggesting that a PGRN-based therapy could significantly supplement existing breast cancer treatment. PGRN-positive expression in cancer specimens correlated with significantly worse overall survival compared to those without PGRN-staining [25]. Recent work demonstrates that PGRN levels are elevated in the serum of patients with breast cancer [28]. In contrast to these studies however, comparing gene expression profiles between 10 tumors from node-positive patients who survived for 5 years after surgery and 10 tumors from those patients who died of breast cancer within 5 years, Asaka et al. [29] concluded that PGRN was significantly downregulated among the patients who died or show worse outcomes and proposed that downregulated expression of PGRN is a prognostic indicator for subdividing node-positive patients into finer groups with "good prognosis".

Ovarian cancer: Ovarian cancer is the deadliest gynecologic malignancy and around 60% of women with it will die from the cancer [30]. In one immunohistochemistry analysis on human primary ovarian carcinoma, effusions, and tissues of metastatic lesions, PGRN protein expression was observed in 95% of ovarian solid tumor specimens, and the expression was detected in all tissue compartments of the ovarian carcinoma, including carcinoma cells, reactive stromal cells, and tumor-associated endothelial cells [31]. In a statistical analysis of *GRN* gene expression in laser microdissected ovarian tumor epithelium, *GRN* expression was observed only in invasive ovarian cancer epithelium and was absent in tumors with low malignant potential (LMP) [32]. Among LMP lesions, PGRN protein expression was, however, occasionally detected in the stromal cells. Miyanishi et al. [33] also reported that immunohistochemical staining of PGRN in the epithelial lesion of ovarian cancer is significantly stronger than that in LMP. PGRN has been investigated as a potential prognostic marker for ovarian cancer. A significantly worse overall survival for patients with high PGRN mRNA expression in ovarian cancers was demonstrated [34]. High PGRN protein expression in ovarian effusion tumor cells correlated to a better overall survival, while elevated PGRN expression in the tumor stromal cells correlated with worse overall survival [31]. Han and et al. [35] studied the relationship between the expression of several serum prediction biomarkers for epithelial ovarian cancer among patients in complete clinical remission. Using receiver operating characteristic and area under the curve analyses to define optimal cut-off points, PGRN levels were significantly associated with worse progression-free survival and overall survival. Elevated PGRN levels at 3 months of clinical remission predicted progression at 18 months.

Uterine cancer: PGRN protein staining was observed in the cytoplasm of tumor cells and stromal cells in endometrial cancer samples [36]. In endometrial cancer, PGRN protein expression was not associated with poor overall survival or known biomarkers of prognosis, including stage and grade. Approximately two thirds of the cancers co-expressed PGRN and ER. Elevated PGRN levels were observed in uterine leiomyosarcomas by immunohistochemical analysis, and the PGRN protein expression positively correlated with histological grades of tumors [37]. Those findings suggested that PGRN might be used to become a specific diagnostic marker for uterus leiomyosarcomas.

Prostate cancer: High levels of PGRN immunostaining were observed in prostate cancer tumor cells both among prostatic intraepithelial neoplasia (PIN) specimens and invasive cancer specimens [38,39]. PGRN was absent or showed low level staining in prostatic glands of normal tissues. However, PGRN expression level has no significant correlation with pathological stage, Gleason score, the status of lymph node metastasis, extraprostatic extension, perineural invasion, surgical margins, and vascular invasion. The elevated levels of PGRN staining in PIN suggest that elevated PGRN production may occur in the earlier stages of prostate cancer development.

Bladder cancer : Monami et al. [40] found that PGRN expression was detectable in both bladder cancer cells and normal bladder urothelium, and suggested that PGRN may play important roles in

carcinogenesis as well as regulation of normal physiological activity. Significantly higher immunostaining of PGRN was observed in invasive bladder tumors as compared with normal bladder tissues [41]. Lovat et al. [41] analyzed PGRN mRNA expression in bladder cancer using microarray database and found that overexpression of PGRN was observed in primary bladder cancers. PGRN mRNA expression levels was higher in high-grade bladder cancer than that of low-grade bladder cancer, and overexpression of PGRN were observed in patients who died after 5 years of follow-up compared with those alive after 5 years of follow-up treatment [41,42]. PGRN levels in voided urine samples from bladder cancer patients revealed that PGRN level was significantly higher in patients with malignant lesions compared to healthy individuals [43].

Kidney cancer: Elevated PGRN levels were detected in renal cell carcinoma samples using Western and immunohistochemical analysis [44]. PGRN expression correlated positively with the histological grades of tumors.

Liver cancer: Overexpression of PGRN was observed in a majority of hepatocellular carcinoma (HCC) patients using immunohistochemical methods on liver tumor specimens [45,46]. Patients with high PGRN content in clinical specimens showed characteristics of aggressive HCC, such as large size of tumor and venous infiltration [46]. PGRN protein is commonly observed in the cytoplasm of HCC tumor cells [46,47]. Cheung et al. [46,48] also reported that strong expression level of PGRN is significantly associated with early recurrence after curative resection and suggested that overexpression of PGRN is a predictive biomarker of poor outcome for HCC.

Esophageal cancer: PGRN protein is highly expressed in both cytoplasm and nuclei of esophageal squamous cell carcinoma cells [49]. Positive staining was also observed in stroma, including sporadic interstitial cells, esophageal glands, and vascular endothelial cells. In well differentiated tumors, positive staining of PGRN is uneven, whereas uniformly strongly positive staining was found in poorly differentiated tumors. PGRN positive staining was almost un-detectable in normal esophageal endothelium. PGRN positive staining was correlated with tumor infiltration depth and TNM classification.

Gastric cancer: Serological identification of tumour antigens by recombinant expression cloning identified *GRN* gene as overexpressed in gastric cancer [50]. PGRN was highly expressed in gastric cancer specimens with 2.3-2.9 folds of difference compared with the adjacent non-cancerous tissues. Using immunohistochemical analysis, PGRN protein expression was almost absent in normal gastric tissues, however 88% of cells were PGRN-positive in gastric cancer biopsy specimens [51]. This may not be specific for neoplasia since increased mucosal PGRN staining was also observed in gastritis [51].

Laryngeal cancer: Using immunohistochemical staining on samples from primary laryngeal cancer and adult laryngeal papilloma as well as laryngeal leukoplakia, PGRN protein expression was found to be significantly higher in the cytoplasm of laryngeal squamous cell carcinomas (LSCC) than those of the other lesions [52]. Overexpression of PGRN mRNA levels was also observed in LSCC tissues.

Lung cancer: PGRN was investigated as a new candidate lung cancer biomarker by one large cohort study but was not found informative as a lung cancer biomarker [53].

Brain cancer: PGRN is consistently overexpressed in human glioblastomas tumors compared with normal brain samples [54]. Using microarrays, Martert et al. [55] investigated gene expression profiles between primary human glioblastoma multiforme (GBM) tumors and normal brain tissues, and found that PGRN is upregulated in GBM tumors. PGRN expression was detected in 36.7% of meningioma tumor samples using reverse transcription-polymerase chain reaction (RT-PCR) [56]. The average size (51.5±5.9 cm^3) of tumors with PGRN expression was significantly larger than the mean meningioma volume (24.9±2.8 cm^3) of PGRN-negative tumors. The mean area of peritumoral brain edema which is associated with malignant development, was significantly larger in PGRN positive tumors than that in tumors with absence of PGRN.

Myeloma and leukemia: PGRN mRNA and protein expression were observed in multiple myeloma (MM) cell lines, and the immunohistochemical analysis showed PGRN positive staining in bone marrow smears from MM patients but no detectable PGRN staining in bone marrow biopsy samples from patients in remission [57]. Larramendy and et al. [58] found that PGRN expression was significantly down-regulated in acute myeloid leukemia as assessed by cDNA microarray analysis.

Infectious agents, progranulin and cancer: In south-east Asia, chronic infection with the liver fluke *Opisthorchis viverrini (O. viverrini)* is a major risk factor for bile ducts cancer or cholangiocarcinoma. Screening genes from the transcriptome [59] or the excretory/secretory proteome [60] of *O. viverrini* that are associated with cancer in humans identified a parasite homologue of human PGRN. PGRN was secreted by the liver flukes and released into bile ducts to stimulate proliferation of biliary epithelial cells to promote host cells to turnover with the potential to increase susceptibility to ductal carcinogenesis [59,60]. Patients infected by *Helicobacter pylori (H. pylori)* have a higher than average risk of developing gastric cancer [61]. *H. pylori* infection was found to induce overexpression of PGRN at both mRNA and protein levels in gastric epithelial cells [51] which may contribute to development of gastric cancers.

PGRN promotes tumorigensis in experimental models

Given that PGRN is often over-expressed in cancers, and that PGRN levels are associated with poor outcome, does PGRN have a functional role in carcinogenesis? Over production of PGRN frequently confers a more aggressive phenotype on cancer cells *in vitro*, as assessed by parameters such as anchorage-independent growth, improved survival or invasion assays (for examples, see refs [34,36,38,40,45,46,57,62-66]). Increasing the production of PGRN in poorly tumorigenic cells often results in a more malignant phenotype following transplantation into mice. The over-production of PGRN in the estrogen-dependent breast cancer cell line MCF7, for example, results in increased tumorigenicity, estrogen-independence and resistance to the estrogen receptor blocking drug Tamoxifen [63,67]. Similarly, PGRN over-production confers greater tumorigenicity upon liver [46], ovarian [68] and endometrial cancer cells [36] when grown as xenografts in mice. SW13 cells, which derive from an adrenal carcinoma, are highly sensitive to the proliferative effects of PGRN [3,65]. The parental SW13 cells are poorly tumorogenic *in vivo*, however by over-producing PGRN they become highly tumorigenic in mice [3]. SW13 cells have defects in the p53 [69] and Rb [70] tumor-suppressor systems, and exhibit little detectable expression of cyclin-dependent kinase inhibitors such as p21 or $p16^{ink4a}$ [69]. Presumably the loss of function of tumor suppressors such as p53 in SW13 cells removes the brakes on the cell cycle, while PGRN provides the acceleration that drives them towards a more malignant state.

Primary cultures of human cells are more difficult to transform by direct gene transfer than rodent cells [71]. Specifically, there is a requirement for genes that prevent senescence (immortalizing genes) to cooperate with genes that activate the RAS-mediated cell signaling pathway and thereby stimulate proliferation [72-74]. In particular, RAS-mediated activation of guanine nucleotide exchange factors for the Ral small GTPases appears critical in the transformation of human cells [75]. Very few genes can be successfully substituted for mutant RAS in these primary cell transformation assays. Examples inhuman ovarian surface epithelial (OSE) cells include the oncogenic epidermal growth factor receptor-family member *HER-2* [76]. Importantly, PGRN is also able to substitute for oncogenic RAS in the transformation of immortalized human OSE cells [33] as well as in human uterine smooth muscles cells [37]. These primary cells [33,37], that were first immortalized by expression of telomerase (*hTERT*) and SV40 large-T antigen (*SV40*) and then forced to express the *GRN* gene at high levels, were strongly tumorigenic when transplanted into athymic mice. Immortalized cells expressing *hTERT* and *SV40*, but that did not over-express the *GRN* gene, were not tumorigenic [37,33]. Similarly the expression of the *GRN* gene on its own did not transform the primary cells. The expression of *SV40* impedes inhibitory control mechanisms on the cell cycle exerted by the p53 and Rb systems, as well as inhibiting the protein phosphatase 2A [77] and prevents mitogen-mediated senescence, while increased expression of the *GRN* gene appears to provide the thrust that drives the immortalized, but non-cancerous cells towards a tumorigenic phenotype. The ability of PGRN to replace oncogenic RAS in transforming immortalized cells suggests that it is a highly oncogenic protein. This interpretation, that PGRN can transform immortalized (pre-cancerous) cells is supported in other systems including the non-transformed but immortal renal epithelial cell line, MDCK, which becomes highly anchorage-independent when engineered to produce elevated levels of PGRN [3].

Just as the over-expression of PGRN promotes a more proliferative and tumorigenic phenotype, the reduction of PGRN mRNA levels may reduce proliferation in vitro and tumor growth *in vivo*. In tissue culture, proliferation and anchorage-independence can be inhibited by targeting PGRN mRNA in breast cancer [78], SW13 adrenal carcinomal cells [3], MDCK kidney epithelial cells [3], laryngeal cancer cells [52], prostate cancer cells [38], ovarian cancer cells [32,79] and liver cancer cells [80]. PC cells, which are a highly tumorigenic murine teratoma-derived cell line, secrete a growth factor, called PC cell-derived growth factor that is identical in structure to murine PGRN [6]. Targeting PGRN mRNA levels in PC cells abolished their *in vivo* tumorigenicity [81]. Lowering the mRNA levels of PGRN in other tumorigenic cell lines, including the breast cancer line MDA-MB-468 [78], the laryngeal carcinomal cell line Hep-2 [52], and liver cancer cells [80] also results in profound inhibition of tumor growth in mice. Monoclonal antibodies against PGRN are effective at inhibiting tumor growth of transplanted liver cancer cells in nude mice, and work by targeting both the growth of the tumor cells directly, but also by suppressing tumor angiogenesis [45]. Taken together, these results suggest that progranulin is both sufficient to stimulate a more malignant phenotype in poorly tumorigenic or immortalized cells; that it is necessary for tumor growth in some aggressive cancers, and that it has promise as a molecular target in the development of novel anti-cancer therapies.

PGRN and the tumor stroma

It is well established that the non-transformed mesenchymal cells that surround the cancer and form the tumor stroma have a crucial role in the growth and metastasis of many cancer types [82]. In most healthy tissues, mesenchymal cells such as fibroblasts or endothelial cells express the *GRN* gene at low or negligible levels [83]. However, in the fibroblasts of the tumor stroma and tumor capillary endothelial cells, *GRN* gene expression may be abundant. This is well documented for ovarian cancers, where approximately half of the tumors examined displayed PGRN-staining in stromal fibroblasts and in two thirds of the tumors capillaries were PGRN positive [31]. This has important consequences since, as noted above the presence of ovarian stromal PGRN correlated with a poorer outcome [31]. In breast cancer cells PGRN stimulated increased production of the angiogenic proteins vascular endothelial growth factor (VEGF) [84,67] and angiopoietin [67]. Esophageal squamous cell carcinomas exhibited a positive correlation between PGRN and VEGF levels, with the levels of both proteins correlating with microvessel density [49]. Blockade of PGRN by monoclonal antibodies in HCC xenografts resulted in a decrease in tumor microvessel density, which was attributed to reduced production of VEGF [45]. PGRN may also have a direct effect on angiogenesis that is independent of VEGF production since it stimulated the proliferation and migration of endothelial cells in culture [20].

McAllister and colleagues have recently proposed a novel mechanism for tumor stroma formation where an initial robust breast cancer mass, the instigator, stimulates the formation of reactive tumor stroma in a second poorly-growing or indolent tumor located at a distant anatomical site [85,25]. The instigating tumors secrete the prometastatic protein osteopontin that activates the migration of a population of $Sca1^{+}cKit^{-}$ hematopoietic stem cells from the bone marrow to the quiescent tumor [85]. Upon taking up residence in the indolent tumor the $Sca1^{+}cKit^{-}$ bone marrow cells secrete PGRN, which in turn activates the growth of a fibroblastic stroma around the quiescent cancer cells [25]. The cancer cells then proliferate under the influence of the recently formed stroma to create new tumor masses [25]. Many of the details of how PGRN contributes to the formation and activity of the stroma remain unclear, but in principle the PGRN-secreting $Sca1^{+}cKit^{-}$ bone marrow cells might initiate the formation of the tumor stroma, while at a later stage intrinsic PGRN production by stromal fibroblasts may supplement or take over the role of the bone marrow cells in promoting and maintaining the growth of the tumor stroma.

PGRN, cell survival and drug resistance

PGRN is a putative survival factor for normal and cancer cells *in vitro*. This may contribute to the overall growth of the PGRN-sensitive tumors, and may complicate cancer therapy since PGRN appears to confer increased resistance to several classes of anti-cancer drugs. PGRN prevents anoikis in cancer cells [65], a form of apoptosis that occurs when cells detach from their basement membrane [86]. Immunoneutralization of PGRN in ovarian cancer cells results in enhanced apoptosis as assessed by increased caspase-3 activation and poly(ADP-ribose) polymerase cleavage [79]. PGRN inhibits metabolic-stress apoptosis in non-transformed fibroblasts [87] and cultured neurons [88,89]. Recent work in *C. elegans* and with macrophage from *Grn* knockout mice revealed that in the absence of PGRN the rate of phagocytosis of apoptotic cells increased [90], suggesting that PGRN may inhibit apoptotic clearance of injured or diseased cells.

Endocrine therapy is a mainstay in the treatment of estrogen receptor (ER)-positive breast cancer, with selective estrogen receptor modulators such as tamoxifen [91] playing an essential role in the treatment of ER-positive breast cancer. Over-production of PGRN in estrogen receptor-positive MCF-7 cells induced tamoxifen-resistance

[63,67]. The activation of mitogen-activated protein kinase (MAPK) signaling pathways in breast cancer cells by the over-production of growth factors or growth factor receptors promotes proliferation without requiring ER-mediated growth signaling [92]. This confers resistance to antiestrogens [93] thereby rendering antiestrogen therapy in effective [92]. In this regard, PGRN markedly increased MAPK activity in breast cancer cell lines [63]. Moreover, PGRN treatment in MCF-7 cells inhibited the tamoxifen-induced down-regulation of bcl-2 [94]. PGRN may in addition interfere with aromatase therapy since PGRN inhibited the efficacy of the aromatase inhibitor letrozole in a human breast cancer cell line [95]. PGRN expression level was significantly increased in letrozole resistant tumor cells compared with letrozole sensitive cancer cells [95]. Other hormone-based therapies may also be sensitive to PGRN production. For example, PGRN decreased apoptosis induced by the synthetic glucocorticoid dexamethasone (which is used in the therapeutic regimen of multiple myeloma) in a dexamethasone-sensitive multiple myeloma cell line [96].

Ovarian cancer cells that constitutively over-produce PGRN were resistant to the platinum containing drug cisplatin [68] (which is used to treat ovarian cancer [97]). However, ovarian cancer stromal cells that were sampled after chemotherapy with platinum drugs tended to show reduced staining for PGRN compared to comparable tissues inspected before treatment [31] suggesting a level of complexity in the drug-PGRN relationship. The recurrence of liver cancer and chemoresistance are huge obstacles to provide curative treatment for patients [48,98,99]. In hepatic cancer stem cells [48], inhibiting the expression of *GRN* gene sensitized the HCC cells to chemotherapy [48], whereas elevated production of PGRN led to resistance to cisplatin and doxorubicin. In chemoresistant HCC cells, PGRN levels were positively associated with expression of the adenosine triphosphate-dependent binding cassette ABCB5 which is a drug transporter in liver cancer cells [48]. The interaction between PGRN and ABCB5 may provide a potential mechanism by which PGRN confers drug resistance. Chemotherapeutic resistance in non-small cell lung cancer (NSCLC) increased with the expression of PGRN [100]. Hu and et al. [101,102] studied the correlation between *GRN* expression in serum or tumor samples and chemotherapeutic sensitivity in NSCLC and found significant higher expression of PGRN in chemoresistant patients than in chemosensitive patients. They concluded that PGRN expression was significantly associated with response of chemotherapy and PGRN might become a biomarker to evaluate chemotherapeutic sensitivity and predict medical prognosis among NSCLC patients.

The mechanism of action of PGRN

A functional receptor for PGRN has not been identified, although chemical cross-linking experiments show that PGRN [103], its constituent grn/epi peptides [104] and TGFe bind in a specific fashion to cell surface proteins [105]. Scatchard analyses indicated that both for PGRN [103] or grn/epi peptides [104] at least two classes of PGRN binding site exist, one of low affinity but high abundance together with other sites of low abundance but high affinity. PGRN binds with sortilin on cell surfaces which is likely to be part of a protein turnover mechanism [106]. PGRN also binds to and inhibits the TNF-receptors [106,107] and associates with the Toll-like receptor-9 [108]. These interactions are important in the regulation of inflammation. Other binding partners for PGRN include non-receptor extracellular matrix proteins such as perlecan [109] and cartilage oligomeric matrix protein [110]. The binding of PGRN to these extracellular matrix proteins modifies the biological action of PGRN either blunting or enhancing the combined proliferative effects of the PGRN-matrix protein pair [110].

PGRN stimulates the MAPK and phosphatidylinositol 3-kinase (PI3K) pathways in cancer cells, as well as in non-transformed fibroblasts and neurons [20,34,38,40,45,57,62,63,65,66,111,112]. Both the MAPK and PI3K signaling systems are essential for PGRN mediated cell division, survival and invasion [65]. Interestingly different cells may show different signaling responses to PGRN, for example, bladder cancer cells show clear activation of MAPK in response to PGRN but little or no PI3K response [38]. Significantly, the bladder cells respond well to the motility and invasive activity of PGRN but not to its proliferative actions [38]. PGRN signaling may interact with integrin signaling pathways through focal adhesion kinase (FAK) [20,65]. In bladder cells PGRN-stimulated the formation of intracellular complexes of MAPK, paxillin and FAK [40] thereby linking the ERK and FAK signaling machinery. FAK is essential for growth factor and integrin-regulated cellular motility (reviewed in [113]). In addition to promoting motility-related signal transduction events, PGRN stimulates the expression of matrix metalloproteinases (MMPs) in cancer cells, including MMP2, MMP9, MMP13 and MMP17 [65,84,114] which probably contributes to the migratory properties of cells stimulated by PGRN. Downstream effects of PGRN signaling include expression of proteins of the cell cycle such as cyclin D1 [62,63,114], and cyclin B [62], the phosphorylation of other signaling intermediates such as glycogen synthase kinase beta [111,112], and the activation of transcriptional regulators such as nuclear factor kappa-B in myeloma cells [96] and JunB in chondrocytes [16].

The activation of the MAPK and PI3K signaling systems are characteristic of all the classic growth factors, however differences between the signaling properties of PGRN and those of conventional growth factors have been postulated. Non-transformed fibroblasts require stimulation by two independent growth factors in order to complete the cell cycle under serum free conditions [115]. One of these signals is provided by an insulin-like growth factor (IGF), while the other signal may be provided by one of a number of growth factors including members of the platelet-derived growth factor (PDGF), fibroblast growth factor (FGF) or epidermal growth factor (EGF) families. Genetically deleting the receptor for IGF-1 prevents the mitotic activity of the IGFs on murine fibroblasts, but also inhibits all other growth conventional factors from stimulating proliferation [116]. PGRN, however, retains the ability to promote cell division in IGF-I receptor negative fibroblasts under serum free conditions, indeed it is the only extracellular protein known to do so [62,117]. It is thought that rather than any ability of PGRN to stimulate distinct signaling pathways it has this property because it stimulates a more prolonged activation of proteins in the MAPK and PI3K pathways than do classic growth factors such as PDGF or EGF [62]. Unlike their wild-type counterparts, IGF-I receptor negative fibroblasts cells (R^- cells) are not transformed by most oncogenes [116], although they are transformed by constitutively active mutant G-protein G-alpha 13 [118], and become sensitive to the transformative activity of SV40 T as they age, possibly due to enhanced expression of the EGF-receptor HER-3 [119]. Whether PGRN signaling also interacts with G-alpha 13 or HER-3 is unknown. The ability for PGRN to promote proliferation in cells such as the R^- cells, that are otherwise refractory to growth factor stimulation or the transformative effects of oncogenes, is worrisome in that it provides a putative pathway through which cells could escape the therapeutic effects of anti-cancer drugs that work by targeting growth

factors and their receptors. A summary of PGRN's role and mechanism in cancer is given in Figure 2.

Although the focus in this article is on PGRN as a mitogen, a cell survival factor and a promoter of invasion, it may have many other biological effects, some of which could contribute to tumor growth. PGRN is anti-inflammatory [17-19], which might in principle effect the host's immune response to developing tumors. Further, PGRN may regulate protein turnover since the over-production of PGRN by HeLa cells, or treatment of the cells with PGRN-conditioned medium, stimulates the formation of more and larger lysosomes [120]. Consistent with these observations, treatments that promote lysosome formation, such as sucrose or the expression of the transcription factor EB, stimulate *GRN* gene expression [120]. PGRN is reported to interact with intracellular proteins such as cyclin-T [121-123] which may modulate transcription. It remains uncertain if these interactions contribute to carcinogenesis, but they clearly provide additional possibilities through which the expression of the *GRN* gene could modulate cell function.

Regulation of *GRN* gene expression

At present there is no evidence that the *GRN* gene is amplified during tumorigenesis, suggesting instead that the elevated expression of the *GRN* gene in cancer cells results from changes in its regulation rather than an increased gene copy number. Mechanisms for the regulation of the *GRN* gene include mRNA stability [124], microRNAs [125-128], the action of RNA binding proteins [129], signal transduction by MAPK signaling [79], nuclear hormone receptors [130], and by physiological parameters such as hypoxia [87,131].

GRN gene expression is stimulated by nuclear receptor hormones such as retinoic acid [124] and, in breast cancer cells [130], endometrial cancer cells [36] and the hypothalamus [132] by estrogen. Importantly, the increase in *GRN* expression that follows exposure to estrogen in breast cancer cells may set in motion a PGRN autocrine growth stimulatory loop [133] since the mitogenic activity of estrogen on MCF-7 breast cancer cells in culture is inhibited by the immunoneutralization of PGRN [63], while enhanced *GRN* expression in breast cancer cells enables them to form tumors independently of estrogen [67].

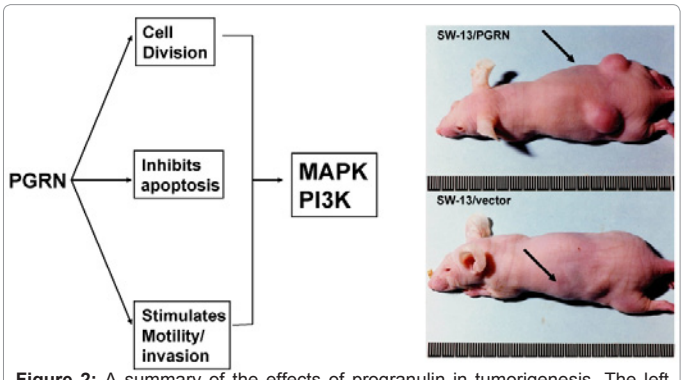

Figure 2: A summary of the effects of progranulin in tumorigenesis. The left panel shows that PGRN stimulates cancer cell migration, survival and mitosis through MAPK and PI3K dependent mechanisms. Although not shown here, progranulin may stimulate the proliferation and activation of tumor resident fibroblasts in the stroma possibly through activating similar biological responses. The right panel shows the growth of SW-13 tumors in athymic nude mice eight weeks after subcutaneous injection into both flanks. Cells that were engineered to over produce progranulin (SW-13/PGRN) formed large tumors (arrow), while control cells (SW-13/vector) gave either small tumor growths (arrow) or undetectable tumor growth. (The scale marker is in cm with 2mm gradations). For details see ref [3].

Mitogens other than estrogen may also stimulate *GRN* gene expression. The proliferative actions of endothelin and lysophospahtidic acid (LPA) on ovarian cells were blocked when PGRN was immunoneutralized, suggesting that PGRN acts as an autocrine signal for these mitogens [79]. Both endothelin and LPA promote the expression of the *GRN* gene [79] through the activation of MAPK by a protein kinase A, calcium and cyclicAMP-dependent mechanism [79]. The up-regulation of *GRN* gene expression by MAPK may be widespread since it is also reported in neuroblastoma cell lines [131] and in gastric mucosa [51] in response respectively to hypoxia or the presence of the gastritis causing bacterium *H. pylori*.

Differentiation agents such as retinoic acid and dimethylsulfoxide increase *GRN* mRNA expression in myelogenous leukemias [124]. This revealed for the first time the importance and complexity of PGRN mRNA stability in *GRN* gene regulation. In a progranulocytic cell line, for example, differentiation agents promoted faster *GRN* mRNA turnover, resulting in a rapid but transient increase in PGRN mRNA following stimulation. In contrast, in a promonocytic cell line identical treatments slowed the rate of PGRN mRNA turnover [124] leading to a slower and more prolonged elevation of the *GRN* mRNA level. Genetic studies on *GRN* in neurodegenerative disease proved that microRNAs (miRs), in particular miR-659 [125] and miR-107 [127] are critical negative regulators of PGRN mRNA levels. This extends to cancer cells, where miRs belonging to the miR-15/107 gene group were found to suppress PGRN mRNA levels in prostate cancer cells [128]. This may be functionally significant since in leukemic and prostate cancers low miR-15/107 correlates with high *GRN* expression [128]. *GRN* gene expression may be negatively regulated by the p53 tumor suppressor system. Restoring a functional p53 to the malignant glioma cell line LN-Z308 which has lost both p53 alleles, results in an decreased secretion of PGRN [134]. Against this however, in HCCs higher *GRN* expression correlated with higher levels of wild-type but not mutated p53 [47] suggesting a complex relationship between *GRN* gene expression and P53. Additional control of PGRN mRNA expression through RNA-binding proteins has recently been established. The dying neurons of patients with *GRN* gene mutations accumulate intracellular aggregates of a cleaved and ubiquitinated DNA-and-RNA binding protein called the TAR DNA Binding protein or TDP-43 [21,22]. The functional relationship between PGRN and TDP-43 in neurodegenerative disease is not well understood, but in normal brain tissue TDP-43 binds to and decreases PGRN mRNA levels [129]. Whether disruptions in the binding of TDP-43 (or similar RNA-binding proteins) to PGRN mRNA have a role in carcinogenesis is unknown.

The proteolysis of secreted PGRN provides an additional control over PGRN levels. PGRN is digested by matrix metalloproteinases (MMPs) including MMP-9 and MMP-14 [135,136] as well as ADAMTS-7 (a member of the "A Disintegrin And Metalloproteinase with Thrombospondin Motifs" gene family) [137]. During inflammation neutrophil-derived enzymes such as elastase and proteinase-3 digest PGRN down to its constituent 6 kDa grn/epi peptides, some of which have biological activity in, for example, the regulation of inflammation [17,138]. This proteolysis is prevented by the secretory leukocyte protease inhibitor (SLPI) which, in addition to inhibiting elastase enzyme activity, physically binds to PGRN and protects it against enzymatic cleavage [17]. Intriguingly there is strong evidence that SLPI and PGRN act in concert to promote ovarian tumor cell mitosis and survival [139,140]. Other proteins in addition to SLPI

may protect PGRN from enzymatic degradation, including extracellular matrix proteins such as cartilage oligomeric matrix protein [141], and serum proteins such as high density lipoprotein [142]. The turnover of PGRN at the cell surface is controlled by Sortilin [106,107]. Sortilin was originally identified as a regulator of lysosomal enzyme trafficking [143] and is critical in controlling extracellular PGRN levels in neuronal cells [106,107,144]. Whether it has comparable functions in cancer cells is at present unknown. Taken together, the levels and activity of PGRN *in vivo* are likely to depend first on the intra- and extracellular factors that regulate PGRN mRNA levels, followed by complex extracellular interactions between PGRN, proteolytic enzymes, and stabilizing proteins such as SLPI that inhibit the proteolysis of PGRN.

Conclusion

PGRN levels are often highly elevated in tumors at many anatomical sites compared to the equivalent normal tissue. PGRN may therefore have potential as a biomarker for disease outcome. PGRN acts as a mitogen, a cell survival factor and a promoter of invasion for a variety of cancer cells. PGRN may have other biological effects that contribute to tumor growth, including the formation of the tumor stroma, the induction of drug resistance, and anti-inflammatory actions. Given the frequency with which PGRN expression occurs in cancers and its tumorigenic biological actions, there is a strong likelihood that studies of the cellular and molecular mechanisms of PGRN action in tumor formation will supply innovative strategies for the development of novel anti-cancer therapies.

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