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ORIGINAL ARTICLE

The over-expression of GH/GHR in tumour tissues with respect to healthy ones confirms its oncogenic role and the consequent oncosuppressor role of its physiological inhibitor, somatostatin: a review of the literature

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Abstract

The interaction between pituitary hormones, GH – PRL, and Growth Factors, GF, plays a fundamental role in the physiological and neoplastic mechanisms of growth, the latter using these factors to a much greater extent compared to the former, with a direct dose-dependent effect on the speed of local or metastatic expansion. In hormone-dependent tumours, the respective male and female sex hormones interact with GH - PRL - GF to sustain the expansion of the tumour. We carried out a review of the literature on the relationship between the expression of GH and GHR in tumour tissues compared to healthy tissues, and on the correlation between this expression and tumour aggressiveness. An over-expression of GH and GHR in tumours was a constant finding. In more than a thousand cases published in various clinical, observational, retrospective studies investigating cervico-facial tumours, lymphoproliferative diseases, breast cancer, prostate cancer, non-small-cell lung cancer, neuroblastomas, oesophageal cancer, glioblastomas, and sarcomas, we constantly found an improvement in objective response, quality of life and survival, compared to conventional oncological protocols, by inhibiting GH and correlated GF using somatostatin.

Abbreaviations:

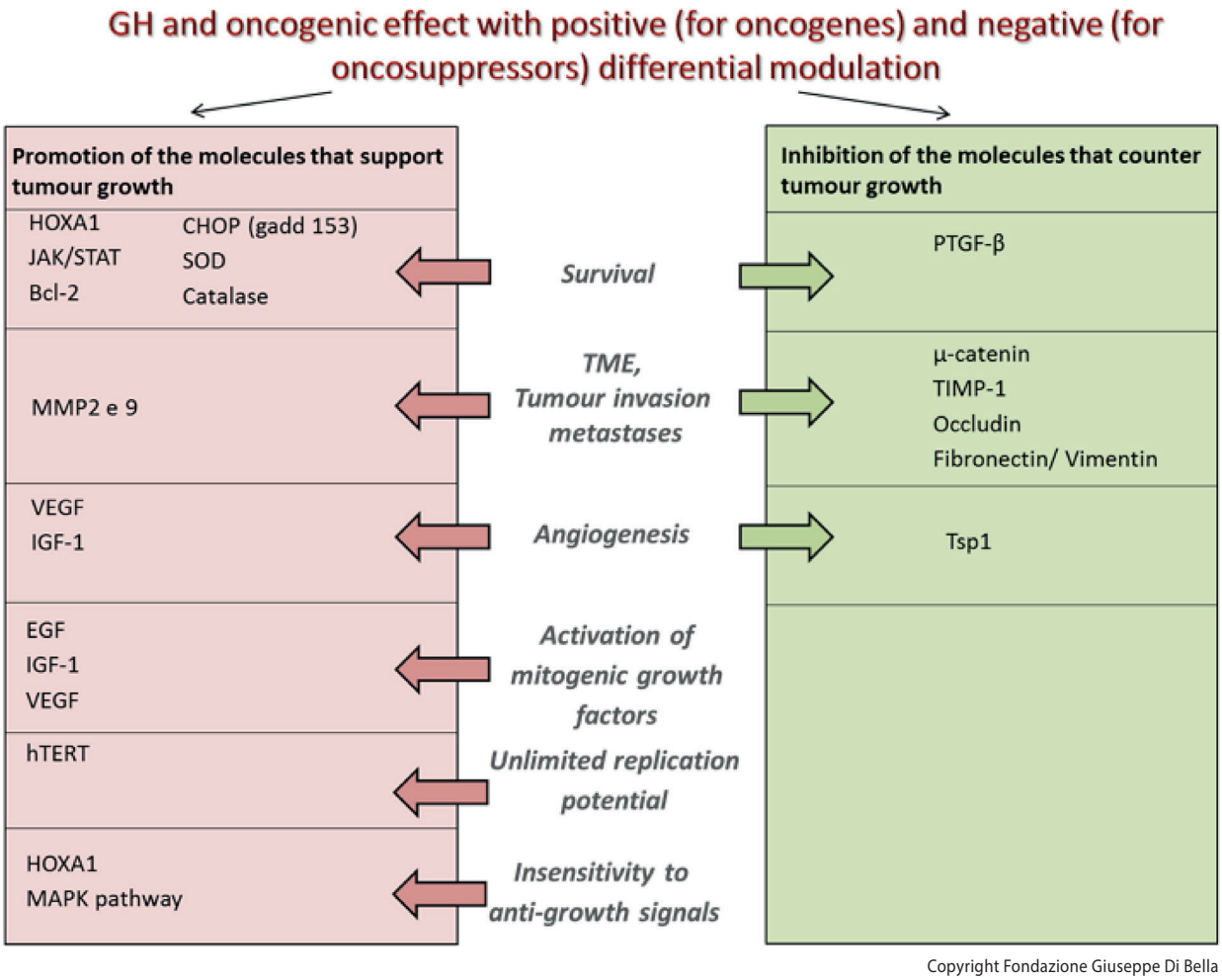
GH	- Growth Hormone
GHR	- Growth Hormone Receptor
PRL	- Prolactin
GF	- Growth Factor
IGF1	- Insulin Like Growth Factor
VEGF	- Vascular Endothelial Growth Factor
EGF	- Epidermal Growth Factor
MAPK	- Mitogen-activated protein kinase
PI3K	- Phosphoinositide 3-kinase
Pit-1	- Pituitary-specific positive transcription factor 1
hTERT	- human Telomerase Reverse Transcriptase
EMT	- Epithelial Mesenchymal Transition
MMP	- Metallo Proteases
TSP1	- Thrombospondin 1
eNOS	- endothelial Nitric Oxide Synthesis
bFGF	- basic Fibroblast Growth Factor
FSH	- Follicle-stimulating hormone
LH	- Luteinizing hormone

INTRODUCTION

Endocrine, biological and biochemical data show the evident primary role of the GH-IGF1 axis in synergy with the other GH correlated growth factors (Fig.1), such as VEGF-A and EGF, together with prolactin (Fig.2). In hormone-dependent tumours, the respective sex hormones, oestrogen and testosterone, interact with GH-PRL-GF. GH is a peptide hormone consisting of 191 amino acids with a weight of 22.005 Da, synthesized, accumulated and secreted by the adenohypophysis. The numerous functions of GH include:

- Regulation of body growth
- Regulation of cell proliferation and differentiation
- Regulation of the metabolism of proteins, lipids and carbohydrates
- Increased protein synthesis in cells with multiple mechanisms including:
- Activation of some carriers of plasma membrane amino acids, causing increased entry into the cytoplasm.
- Transduction of cellular mRNA even without a greater than normal concentration of amino acids
- Increased protein synthesis by ribosomes

GH is the main mediator of the postnatal growth of somatic cells (Le Roith *et al.* 2001), and its effects on cell growth and differentiation are mediated through



ONCOGENES: TFF1 and 3: Trefoil factor; **HOXA1**: Homeobox 1; **MAPK**: mitochondrial protein kinases; **MMP2** and 9: metalloproteases 2 and 9; **Fibronectin and Vimentin**; **JAK / STAT**: Janus kinase proteins and transducing signal proteins and transcription activator; **Bcl-2**: pro-apoptotic protein; **CHOP (gadd 153)**: C / EBP homologous protein; **SOD**: superoxide dismutase; catalase; **VEGF**: vascular endothelial growth factor; **IGF-1**: similar insulin growth factor; **EGF**: growth factor of the epidermis; **h-TERT**: telomerase
ONCOSUPPRESSORS: μ-catenin; TIMP-1: tissue inhibitor metalloprotease; **occludin; PTGF-β**: transforming placental growth factor; **Tsp-1**: antiangiogenic protein thrombospondine

Fig. 1. Growth hormone interacts with many genes (oncogenes and oncosuppressors) that are part of growth different mechanism. At the middle of image (survival, TME, angiogenesis..) different mechanism of growth; in red factors and enzymes that are promoted by GH to induce tumour; in green factors that are inhibited by GH.

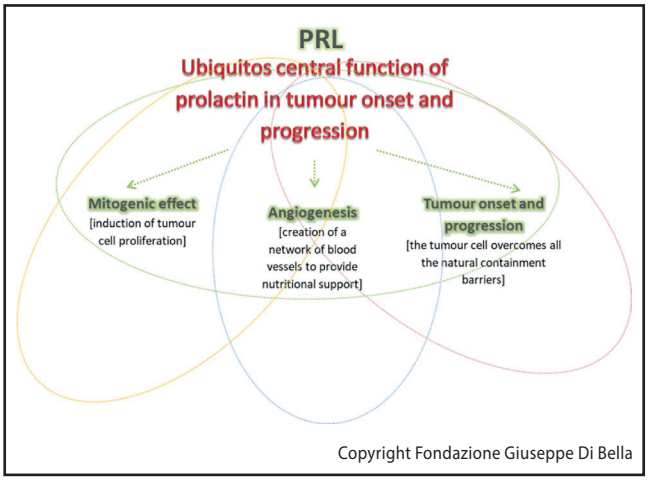


Fig. 2. Prolactin has a central role in tumour onset and progression through mitogenic effect and angiogenesis also.

interaction with its receptor (GH) (Le Roith *et al.* 2001; Zhu *et al.* 2001) which activates signal transduction pathways critical for cell growth and survival including signal transductors (JAK-2/STAT), the cascade of the mitogen-activated protein kinase (P44/42 (MAPK), and of the phosphoinositide 3-kinase (PI3K) (Le Roith *et al.* 2001; Zhu *et al.* 2001). The receptor of GH belongs to the large family of cytokine or hematopoietic receptors, which do not contain the tyrosine-kinase domain in their cytoplasm region.

The family of the GH/cytokine receptors (Class I) and the family of the interferon receptors (Class II) share structural characteristics, recently identified, and common signal transduction pathways. In addition, both classes of receptors are associated with various members of the family of Janus tyrosine kinases and activate a new family of transcription factors, known as transcription transducers and activators, which relate ligands to activation of gene expression (Goffin & Kelly, 1996).

To understand the actions of GH it is necessary to know the structure and expression of its receptor. The cloning and sequencing of the GH receptor showed that this receptor is not homologous to other receptors with a known function, but the GH – Prolactin's receptor homology is a scientifically and clinically important factor due to its therapeutic repercussions. A widespread distribution with variable concentrations of GHR was observed in many types of normal and tumour cells, with a marked and significant prevalence in tumour cells in proportion to the proliferative index and to the invasive and metastasizing capacity (Lincoln *et al.* 1998).

The expression of the IGF-1 gene is regulated differently by the GH in different tissues (Lowe *et al.* 1987). The dependence on GH of IGF-1 has been extensively confirmed in humans. In patients with complete GH deficiency, the levels of IGF-1 are always decreased, with the lowest levels found in patients with Laron dwarfism in whom the GHR is missing (Daughaday & Trivedi 1987) The administration of GH causes an increase of IGF-1 in GH-responsive patients. However, the co-localization of IGF-1 and GHR is not necessary since the role of GH in the tumour cells may be to regulate the function of the mature cells rather than to promote cell proliferation by local synthesis of IGF-1. These two hormones do not always act in harmony; in some tissues the synthesis of IGF-1 is independent of GH (Hynes *et al.* 1987), despite the fact that these tissues possess GHR, as shown by a powerful mitogenesis independent of IGF-1 in response to GH (Rabinovitch *et al.* 1983). More in-depth studies are required to establish whether, and in what conditions and in which tissues GH can act independently of the synthesis of IGF-1 in human tumour cells.

A high concentration of the receptor was also constantly observed in cell lines from tissue in the exponential growth stage. The protein that binds the GH in the nucleus has the same binding sites as cytoplasmic and membrane GHR. The GH / IGF-1 axis is the main mediator of somatic growth and during infancy plays an essential role in the development of the mammary gland, regulating cell proliferations, differentiation and apoptosis (Kleinberg 1997). The determining role of the GH / IGF-1 axis (Laban *et al.* 2003), and its close receptor, functional, and proliferative interaction with the other pituitary secretion, prolactin, with other GH-correlated growth factors, such as VEGF and EGF, is therefore scientific evidence in tumoral genesis.

Review of the clinical and experimental studies confirming the mitogenic properties of GH and the relative biochemical and molecular mechanisms of action

IN BREAST CANCER

- The expression of GHR is associated with the malignant transformation of the mammary gland in a dose-dependent manner (Lincoln *et al.* 1998);
- Human recombinant GH increases proliferation in breast cancer (Conte *et al.* 1990);
- There is an increase in incidence and aggressiveness of secondary malignant tumours, including breast cancer, in patients on the Childhood Cancer Survivor Study treated with rhGH to maximize growth (Rutter & Rose 2007; Neglia *et al.* 2001; Ergun-Longmire *et al.* 2006; Sklar *et al.* 2002);
- IGF-1, the mitogen that acts as an intermediary in the action of GH, is over-expressed in breast cancer and the bond with IGF-1 is higher in breast cancer tissue than in normal adjacent tissue; (Arteaga & Osborne 1989; Yee *et al.* 1989)
- The expression of the protein and of mRNA of GHR has been identified in human breast cancer (Mertani *et al.* 1998; Decouvelaere *et al.* 1995);
- Cell lines of breast cancer produce and secrete IGF-1 (Huff *et al.* 1986);

- Human recombinant GH increases proliferation in breast cancer (Conte *et al.* 1990);
- An increase in levels of HGHR mRNA in samples of human breast cancer with respect to adjacent normal tissue (Gebre-Medhin *et al.* 2001);
- RT-PCR and Western blot analysis showed the expression of GHRH and its receptor in breast cancer, and antagonists of GHRH inhibit the growth of tumors (Schally &Varga 2006);
- The proteins that regulate the secretion of hGH by the hypophysis are implicated in breast cancer (Chatzistamou *et al.* 2004; Kahán *et al.* 1999);
- The induction at the forced expression of Pit-1 increases the expression of GH mRNA and proliferation in human breast cancer cells (Gil-Puig *et al.* 2005);
- GH favours the immortalization of mammary epithelial cell lines through the increase of the levels of mRNA and of proteins of catalytic subunit of telomerase, hTERT (Emerald *et al.* 2007; Dimri *et al.* 2005; Stewart &Weinberg 2006);
- autocrine GH inhibits the mechanisms of anchorage and adhesion in breast cancer cells and of tumour growth in vitro (Kaulsay *et al.* 1999; Mukhina *et al.* 2004);
- the expression of autocrine hGH therefore satisfies the criteria of being considered as an oncogene for human breast cancer (Lincoln *et al.* 1998);
- the expression of GH in MCF-10A cells leads to filling of the lumen due to upset of the normal mammary gland architecture and of the proliferative order (Zhu *et al.* 2001);
- GH increases the metastasizing of breast cancer by interruption of the cell-cell contact and increase in cell migration and invasion (Mukhina *et al.* 2004);
- IGF-1 (GH-correlated) is over-expressed in breast cancer (Yee *et al.* 1989);
- GH is higher in breast cancer tissue with respect to adjacent normal tissue (Arteaga &Osborne 1989);
- Recent studies showed that the expression of GH can increase telomerase activity and extend the replicative ability of a primary mammary epithelial cell line (Emerald *et al.* 2007);

IN GENERAL IN TUMORS

- GH significantly increases the expression of the proto-oncogene c-myc (Murphy *et al.* 1987);
- At high concentrations, GH directly accelerates the growth of osteogenic sarcoma, at the same time inducing high levels of somatomedin (Ward *et al.* 1987; Ratner & Hare 1983);
- GH accelerates the growth of multiple myelomas (Hägg *et al.* 1988);
- the GHI/GF1 axis is highly represented in human lung cancer tissue obtained immediately after surgery, compared to healthy surrounding lung tissue (Minuto *et al.* 1986);
- high plasma levels of GH are documented in numerous human tumours (Adamson *et al.* 1980; Andrews 1983);
- an increased concentration of GH was demonstrated in bone tumours (Ratner & Hare 1983);
- a high and significant concentration of GH was found in multiple myelomas (Hägg *et al.* 1988);
- in human lung cancer tissue obtained immediately after surgery, GH-dependent concentrations of IGF-1 GH were found to be decidedly higher than in the normal surrounding tissue (Minuto *et al.* 1986);
- an increase in incidence and aggressiveness of Hodgkin's lymphoma and colon-rectal cancer was seen in patients treated with hGH during infancy or early adulthood (Swerdlow *et al.* 2002);
- IGF-1 is over-expressed in tumours of the colon (Sklar *et al.* 2002);
- Various clinical studies and case reports have shown an increased incidence of polyps, adenomatosis of the colon and cancer of the colon in patients with acromegaly (Ron *et al.* 1991; Ziel & Peters 1988; Pines *et al.* 1985; Brunner *et al.* 1990);
- The expression of GHR was shown in cell membrane, cytoplasm, the nucleus of normal tissues and in greater concentrations in tumour cells (Lincoln *et al.* 1998);
- The expression of autocrine GH promotes cell proliferation (Kaulsay *et al.* 1999);
- Autocrine GH is the first example of a human gene that can both potentially immortalize and oncogenically transform the human epithelial cell (Lincoln *et al.* 1998);
- GH promotes the phenotypic conversion of cells from epithelial to mesenchymal morphology (EMT) with the acquisition of a migratory and invasive epithelial-mesenchymal phenotype, through the down regulation of plakoglobin, re-localization of E-cadherin to the cytoplasm, and a greater activity of matrix metalloproteases 2 and 9 (MMP) (Mukhina *et al.* 2004; Sommers *et al.* 1994; Thiery 2002);
- GH promotes the migration of endothelial cells and angiogenesis, and increases the levels of VEGF-A mRNA and (Brunet-Dunand *et al.* 2009) (Fig. 3) by down-regulation of thrombospondin 1 (TSP1), inhibitor of the angiogenic phenotype (Lawler & Detmar 2004);
- The transduction of GH leads to a significant induction of numerous genes of angiogenic inductors such as endothelial nitric oxide synthesis (eNOS), and of angiogenic growth factors such as VEGF basic fibroblast growth factor (bFGF), while immunohistochemical analysis revealed an increase in capillary density and cell proliferation (Kusano *et al.* 2007);
- the GH/IGF-1 axis has a protective effect against radiation-induced programmed cell death (Jameel *et al.* 2004; Perry *et al.* 2006);
- GH inhibits apoptosis pathways, negatively regulating cell growth stop genes such as gadd153 / CHOP

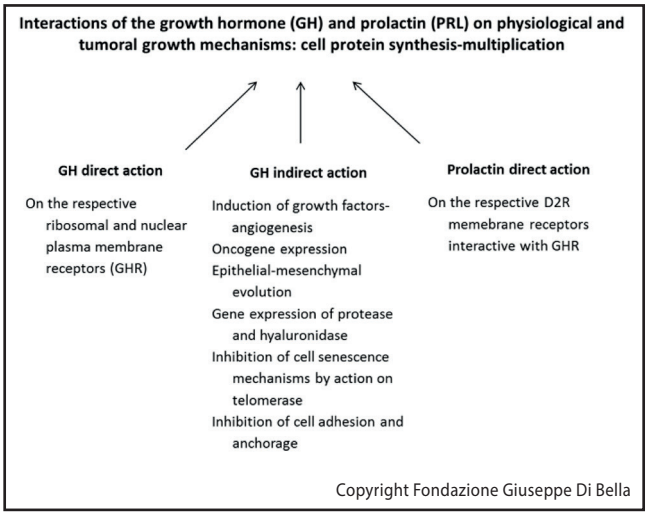


Fig. 3. Cooperation between direct and indirect actions of GH and prolactin on tumour induction.

(C / EBP), thus protecting tumour cells also from oxidative stress (Zhu *et al.* 2005)
- GH is radioprotective in various breast cancer cell lines (Brunet-Dunand *et al.* 2009);
- In addition, the over-expression of HGHR mRNA and of proteins predicts the response to radiotherapy in rectal cancer (Wu *et al.* 2006);

The concept of the binary proliferative GH/IGF1 axis can be extended to the quaternary GH/IGF1/PRL/ER axis in breast cancer (Fig.3). The mitogenic synergism of the growth hormone with the Insulin-like growth factor, with Prolactin and testosterone in male prostate cancer and with oestrogens in female reproduction system tumours and breast cancer has now in fact been confirmed (Gallego *et al.* 2001). We believe it is useful to highlight the fact that most of the mitogenic effects of GH in somatic cells are mediated not only by hepatic IGF-1 but also by induction of the expression of other growth factors such as EGF (Vacas *et al.* 2016) and VEGF-A (Brunet-Dunand *et al.* 2009). The bond between GH and GHR, as well as the documented positive regulation of IGF1, EGF and VEGF, activates the signal transduction of various pathways including JAK-2/STAT, MAPK, and PIK3.

It is a significant documented fact that subjects with acromegaly have an increased risk of colon-rectal cancer (Jenkins *et al.* 2006). High serum levels of GH (Emerman *et al.* 1985) and consequently of IGF-1 (Laban *et al.* 2003), (Yakar *et al.* 2005; Khandwala *et al.* 2000) have been observed in 40% of patients with breast cancer. In other tumours too, such as lymphoproliferative diseases, the expression of GHR has been observed, consistently with its presence in cultivated human lymphocytes (IM 9 cell line) shown with radio-marked ligands (Hughes & Friesen 1985). The GH/IGF-1 axis also modulates the immune system, although the way in which this interaction takes place and the proportional relationships have not been clarified. GH directly regulates the function of the lymphocytes through its receptor (Lesniak *et al.* 1987), or with an action mediated by IGF-1 (Kozak *et al.* 1987). The increase of GHR in melanocytes, nevi, primary melanomas and metastatic melanomas is evidence of its activation of the tumour's progression in these diseases. The expression of GHR in benign prostate hyperplasia and in carcinoma of the prostate is proportional, with a dose-dependent relationship with the aggressiveness and proliferative index of these cell clones (El Etreby & Mahrous 1979; Sinowatz *et al.* 1991; Bengtsson *et al.* 1988).

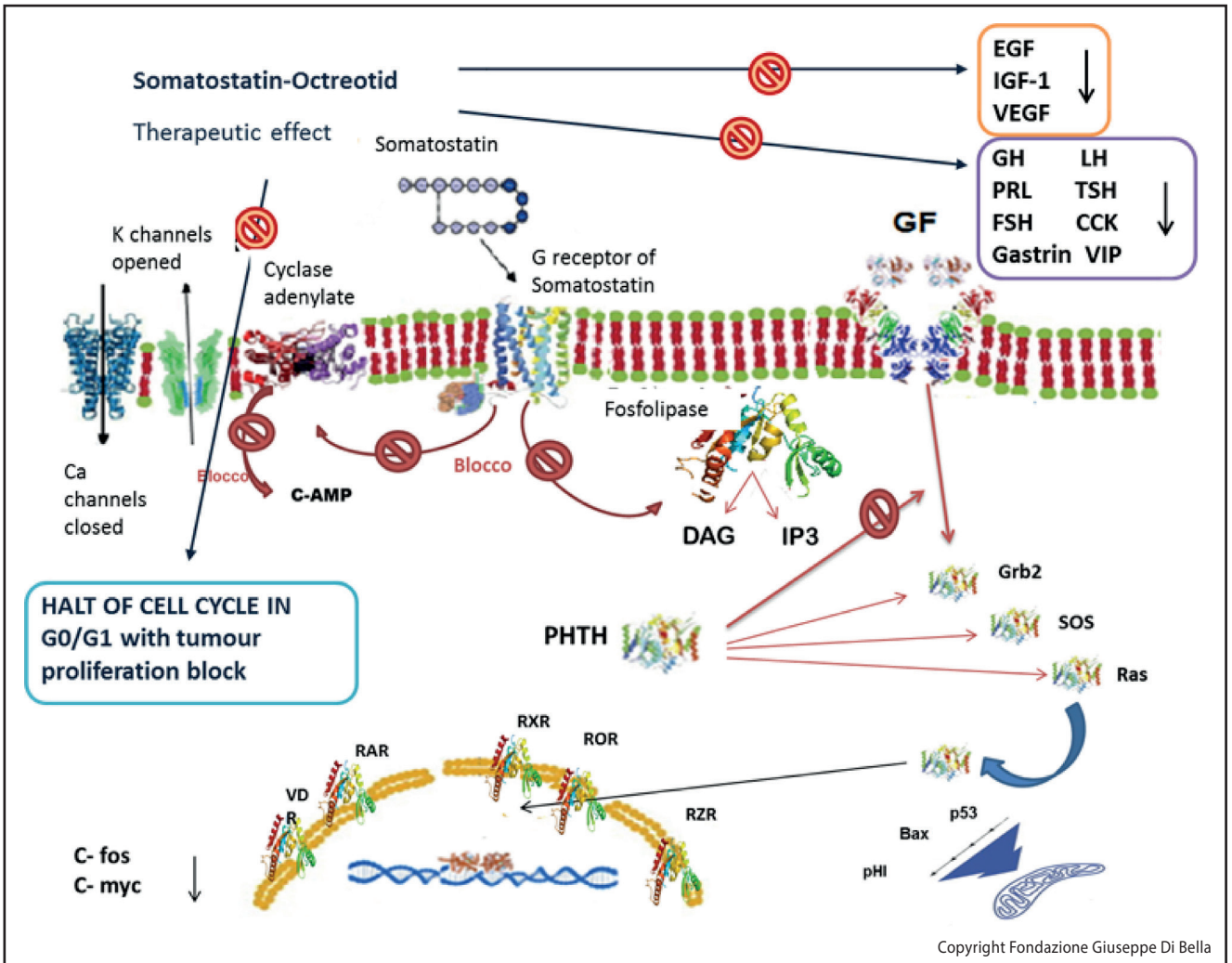
Clinical, retrospective observational studies confirmed the evident anticancer efficacy of the inhibition of GH in breast cancer (Di Bella *et al.* 2013a), as in many other non-neuroendocrine tumours, such as sarcomas (Di Bella *et al.* 2015b), glioblastomas (Di Bella *et al.* 2015a), neuroblastomas (Di Bella & Colori 2009a), cervico-facial tumours (Di Bella *et al.* 2012a), oesophageal tumours (Di Bella &Madarena 2009), non-small cell lung cancer (Norsa & Martino 2006), chronic lymphatic leukaemia (Todisco . 2009; Di Bella *et al.* 2012), and Hodgkin and Non Hodgkin lymphoma (Todisco *et al.* 2001), by means of its physiological antidote, somatostatin.

CONCLUSIONS

These experimental and clinical data, consistent with the biological function of GH, provide further confirmation of the oncogenic induction of its over-expression and the dose-dependent relationship between the extent of the GH/IGF1/GHR expression and the proliferative and aggressive characteristics of the tumour clones (Wu *et al.* 2011). Through the differential regulation of the gene expression, autocrine GH also regulates vital molecular and biochemical mechanisms such as cell growth and survival, migration and invasion, epithelial-mesenchymal transition (EMT), replication potential and oncogenic transformation. The genes that autocrine GH regulates positively or negatively to induce oncogenesis are known. (Perry *et al.* 2008).

This scientific evidence therefore fully validates the rationale for the generalised use in oncotherapy of somatostatin which acts indifferently and equally on pineal and autocrine GH, regardless of the presence of SSTR in the tumour cells. Since GH and correlated growth factors are over-expressed in all tumours, albeit to different extents, with activation of numerous proliferative and angiogenic signalling pathways, the negative regulation of GH by means of somatostatin is logical, and is extended to the correlated growth factors, as widely documented in the literature (Fig.4).

The generalised anticancer use of somatostatin is therefore justified, since it antagonizes the common denominators, and causal factors of all tumours, and the over-expression of the Growth hormone – corre-



C-AMP: Cyclic Adenosine Monophosphate; **SST**: Somatostatin; **DAG**: Diacylglycerol; **IP3**: Inositol triphosphate; **GF**: Growth Factor; **PHTH** Phosphatase; **Grb2**: protein 2 binding growth protein; **SOS**: nucleotide exchange factor; **EGF**: epidermal growth factor; **IGF-1**: similar insulin growth factor; **VEGF**: vascular endothelial growth factor; **GH**: growth hormone; **PRL**: prolactin; **FSH**: stimulating follicle hormone; **LH**: luteinizing hormone; **TSH**: thyrostimulant hormone; **CCK**: cholecystokinin; **VIP**: intestinal vessel peptide; **Ras**: proteins related to cell growth and proliferation mechanisms; **c-fos, c-myc**: proto-oncogenes; **RAR**: retinoic acid receptors; **ROR**: orphan retinoid-MLT receptors; **RZR**: Z retinoid-MLT receptors; **VDR**: vitamin D receptors; **RXR**: Retinoic acid receptors; **P53**: tumor suppressor; **Bax**: pro-apoptotic protein; **pHI**: intracellular PH

Fig. 4. Somatostatin's pathway (and its analogous, octreotid). These molecules block survival mechanisms (c-AMP, Fosfolipase, Growth Factors…) arresting cell cycle in G0/G1.

lated Growth Factors axis. In breast cancer, the close receptor interaction of GH with prolactin and the functional interaction with oestrogen lead, in a multi-therapy setting, to synergic inhibition of GH-GF with somatostatin/octreotide (Fig.4), and of prolactin with D2R agonists (Fig.5), and estrogenic interaction by means of FSH-LH analogues and aromatase inhibitors, with decided progress in the treatment of these tumours (Di Bella *et al.* 2013b; Di Bella 2011). In cases of breast cancer monitored at 5 years, this biological multitherapy synergistically reinforced by the cytostatic, differentiating, immunomodulating and trophic functions of Melatonin, Solution of retinoids in vitamin E, vitamin D3 and vitamin C, (Di Bella Method), without toxic effects, significantly improved quality of life, objective response and survival compared to the same tumour stages of breast cancer treated with conventional oncological protocols (Di Bella *et al.* 2013b; Di Bella *et al.* 2018; Di Bella 2011; Di Bella & Colori 2009b) . In the same way, in prostate cancer the synergic inhibition of GH-GF with somatostatin/octreotide, of prolactin with D2R agonists, and the androgenic block by means of bicalutamide and FSH-LH analogues allowed progress in the treatment of these tumours (Di Bella *et al.* 2013b). We draw attention to these concepts and these data with the intention of improving the prognosis of tumours by making use of this currently undervalued scientific evidence.

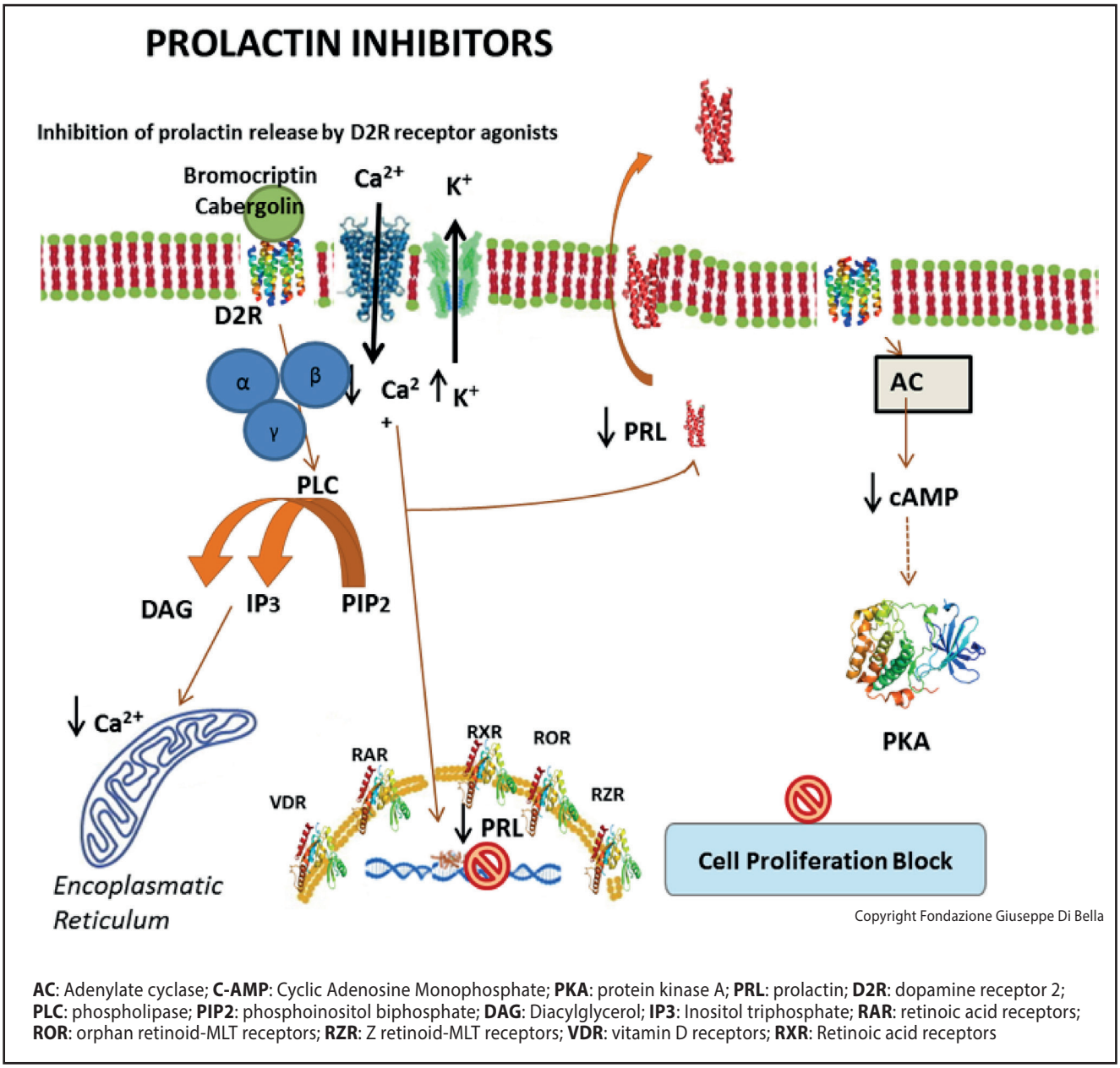


Fig. 5. Bromocriptin and cabergolin are inhibitors of prolactin' release, binding D2R receptor. The activation of D2R receptor induces different mechanism in cell until proliferation block.

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