Prostate Cancer and Prostatic Diseases (2012) 15, 320 – 328

www.nature.com/pcan

REVIEW

Growth/differentiation factor-15: prostate cancer suppressor or promoter?

P Vaňhara[1], A Hampl[1,2], A Kozubík[3,4] and K Souček[2,3]

Deregulation of expression and function of cytokines belonging to the transforming growth factor-β (TGF-β) family is often associated with various pathologies. For example, this cytokine family has been considered a promising target for cancer therapy. However, the detailed functions of several cytokines from the TGF-β family that could have a role in cancer progression and therapy remain unclear. One of these molecules is growth/differentiation factor-15 (GDF-15), a divergent member of the TGF-β family. This stress-induced cytokine has been proposed to possess immunomodulatory functions and its high expression is often associated with cancer progression, including prostate cancer (PCa). However, studies clearly demonstrating the mechanisms for signal transduction and functions in cell interaction, cancer progression and therapy are still lacking. New GDF-15 roles have recently been identified for modulating osteoclast differentiation and for therapy for PCa bone metastases. Moreover, GDF-15 is as an abundant cytokine in seminal plasma with immunosuppressive properties. We discuss studies that focus on the regulation of GDF-15 expression and its role in tissue homeostasis, repair and the immune response with an emphasis on the role in PCa development.

Prostate Cancer and Prostatic Diseases (2012) **15,** 320–328; doi:10.1038/pcan.2012.6; published online 28 February 2012

Keywords: growth/differentiation factor-15; macrophage inhibitory cytokine 1; transforming growth factor-β

INTRODUCTION

Cancer does not merely affect a single cell or an enclosed population of homogenous tumor cells. Thus, studying processes that modulate the tumor microenvironment, particularly the relationship between cancer or stromal cells and the immune system, has immense clinical potential. New findings that describe potential methods to modulate particular cell populations can offer novel strategies for cancer prevention and therapy. Cytokines represent important signaling molecules that regulate the fate of both cancer cells and other cell types within the tumor microenvironment. Several examples exist in clinical practice, where revelation regarding the role of a particular cytokine in cancer progression led to a novel anti-cancer therapy design and significantly improved its efficiency.[1]

Deregulation of expression and function of cytokines belonging to transforming growth factor-β (TGF-β) family is often associated with cancer.[2] Thus, cytokines in this family represent potential candidates for drug targeting. However, the detailed cancer-related functions of several TGF-β family members are still not clear. One of these, growth/differentiation factor-15 (GDF-15), is a divergent member of TGF-β family.[3] This cytokine has been proposed to possess immunomodulatory functions and its high expression is often associated with cancer progression (for review see[4]). However, studies clearly demonstrating its function in tissue development and hematopoiesis and cancer progression have not been conducted. More detailed elucidation of the physiological function of GDF-15 may lead to innovative new cancer treatment strategies to benefit future patients.

GDF-15 SEQUENCE AND STRUCTURE

GDF-15 (synonyms: macrophage inhibitory cytokine 1, non-steroidal anti-inflammatory drug (NSAID) activated gene-1, prostate differentiation factor, placental bone morphogenetic protein; placental TGF-β) was discovered simultaneously by several groups[3,5–8] at the end of 1990s and is localized to chromosome 19 in the region p13.11. Its DNA sequence is 2746 bp long and consists of two exons separated by a single intron.[9] There are at least two GDF-15 alleles, which were identified and characterized in detail by Breit's group.[10] The polymorphism, labeled H6D, consists of a single C-G transversion in exon II at 2423 bp, resulting in a switch from histidine to aspartic acid at codon 202 of the mature protein. This substitution changes the biochemical properties of the mature protein and may alter GDF-15 interactions. The H6D form of GDF-15 has potential clinical relevance, as several studies indicated better prognosis in prostate cancer (PCa) patients carrying the G allele (H6D protein) than those with wild-type GDF-15.[11,12] According to the Hardy–Weinberg equilibrium, the genotype frequencies in the healthy population were estimated to be 54% for homozygotes containing only histidine (alleles CC), 7% for aspartic acid homozygotes (GG) and 39% for heterozygotes (CG).[10]

The unprocessed translated form of GDF-15 (pre-pro-GDF-15) is 308 amino-acids (aa) long, including the signal sequence (29 aa), the propeptide (167 aa) and a mature protein (112 aa), which contains a cysteine knot typical for the TGF-β family. The N-terminal region (28 aa) of the proprotein was shown to be involved in the endoplasmic reticulum quality control and

[1]Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, Czech Republic; [2]Centre of Biomolecular and Cellular Engineering, International Clinical Research Center, St Anne's University Hospital Brno, Brno, Czech Republic; [3]Department of Cytokinetics, Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno, Czech Republic and [4]Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic. Correspondence: Dr K Souček, Department of Cytokinetics, Institute of Biophysics, Academy of Sciences of the Czech Republic, Královopolská 135, Brno, CZ-612 65, Czech Republic.
E-mail: ksoucek@ibp.cz
Received 16 November 2011; revised 10 January 2012; accepted 13 January 2012; published online 28 February 2012

subsequent proteasomal degradation of the incorrectly folded GDF-15 precursor.[13] The generation of the biologically active form requires removal of the hydrophobic signal sequence followed by disulfide-linked dimerization of GDF-15 monomers and a final cleavage by furin-like protease at the canonic RXXR site (RRAR, position 196). This process generates the C-terminal form of GDF-15 with a molecular weight of ~20 kDa that subsequently enters secretion pathway.[3] Further posttranslational modifications utilize a potential N-glycosylation consensus site at 70 aa (NQS); however, the phosphorylation of mannose residues targeting TGF-β proteins to lysosomes has not been found,[13] suggesting the N-glycosylation of GDF-15 is probably not critical to enter the secretory pathway as in case of TGF-β.[14] The secreted mature protein is a 25-kDa dimer cleaved from the 62-kDa intracellular precursor.[6]

The production of biologically active GDF-15 is remarkably complex, and variability in the pool of available GDF-15 forms was suggested to be involved in modulating the tumor microenvironment when differences in GDF-15 expression between malignant and normal tissues were described. The premature proprotein is produced preferentially by cancer cells over normal tissues, and the preprocessed or mature GDF-15 forms have been suggested to be differentially deposited in extracellular matrix (ECM).[15] Current data supports the assertion, that it is the propeptide that mediates interactions of pro-GDF-15 with ECM.[15] Therefore, the tissue availability of GDF-15 depends on ECM degradation, histological composition and architecture or presence of enzymes capable of conversion of pro-GDF-15 to GDF-15, implying a regulation similar to TGF-β.[15,16] However, direct binding of GDF-15 to latent TGF-β-binding protein-1, which normally sequesters TGF-β and induces binding to the ECM, has not yet been described. Interestingly, GDF-15 stimulates the expression and surface stabilization of matrix metalloproteinases (MT1-MMP) in different cell types, including breast cancer (MCF-7) or human embryonic kidney cells (HEK293) that are sensitive to GDF-15-induced growth arrest. Because GDF-15 is simultaneously a substrate for MT1-MMP, the inhibitory effects on cancer cells are abrogated after MT1-MMP stimulation by GDF-15. This feedback circuit may have particular significance for ECM remodeling in the tumor environment, tissue permeability in metastatic spreading and for tumor growth.[16] The availability of mature GDF-15 or activation of the proprotein by ECM-deposited and microenvironment-regulated proteinases increases the complexity of the GDF-15 regulatory network in a manner tightly linked to the cell and tissue microenvironment, especially under pathological conditions.

GDF-15 IN TISSUE HOMEOSTASIS AND REPAIR

Expression of GDF-15 is tightly associated with conditions of stress or damage in tissues, indicating its role in tissue regeneration or healing, as documented in numerous cases, such as for myocardium.[17–23] Because GDF-15 is not normally expressed in the healthy heart, it is rapidly upregulated upon stress or with markers of heart damage, such as pressure overload, inflammation, oxidative stress or ischemia, suggesting an anti-apoptotic or protective function during heart failure, arterial hypertension or other cardiovascular insufficiencies. GDF-15 can also offer clinical prognostic information. For example, high-GDF-15 levels in plasma indicate worsened outcomes for particular cardiac malfunctions.[19,20,22–25] Biological functions of GDF-15 for tissue regeneration in myocardium have not been satisfactorily clarified so far; however, the polymorphonuclear leukocytes[26] or macrophages[27] infiltrating the effected site have been proposed as target populations for GDF-15. The pathological accumulation of polymorphonuclear leukocytes in infarcted myocardium may be prevented by GDF-15 secretion, as it reduces polymorphonuclear leukocyte adhesion by the inhibition of integrin β2 and small GTPase signaling *in vitro*.[26] Along with outside-in signaling, GDF-15-mediated signal transduction in cardiomyocytes involves the canonical SMAD pathway (SMAD2/3). Both actions not only prevent pathological changes in tissue architecture, such as cardiac hypertrophy and ventricular dilation, but can also inhibit an inappropriate immune response. Under hypoxic conditions in human umbilical vein endothelial cells *in vitro*, treatment with GDF-15 significantly enhances HIF1α-mediated expression of VEGF and also stabilizes the p53-MDM2 complex leading to ubiquitination and subsequent degradation of p53.[28] Besides cardiovascular tissue, elevated GDF-15 expression has been found in patients with rheumatoid arthritis,[29] congenital anemia[30,31] and metabolic disorders, such as obesity, diabetes mellitus or preeclampsia.[32,33] In cases of ineffective hematopoiesis, GDF-15 is likely involved in iron metabolism and erythrocyte differentiation.[34] GDF-15 overexpression was measured after mechanical liver or kidney injury [35] and was capable of inducing the renewal of specific cell populations, including renal acid-secreting collecting duct cells.[36] In mice, GDF-15 may function as a neurotrophic and neuroprotective cytokine, as GDF-15 knock-out mice show postnatal loss of motoneurons in spinal cord and brainstem motor nuclei and dorsal root ganglionic sensory neurons in superior cervical ganglion.[37] In this study, Strelau *et al*.[37] also demonstrated that GDF-15 produced by Schwann cells promotes the survival of axotomized dopaminergic neurons both *in vivo* and *in vitro*. Further investigation in ischemia-induced brain lesions showed strong and rapid induction of GDF–15 mRNA in neurons and partially in microglial cells; however, a comparison of identically lesioned GDF-15-knock-out and wild-type mice did not reveal a significant difference in infarct area, suggesting a role for GDF-15 in post-lesion adaptation and regeneration rather than general protection or neuronal tissue nutrition.[38]

In addition to tissue regeneration and repair, GDF-15 is involved in human embryonic development, as it is highly expressed in the placenta during pregnancy, and low levels of GDF-15 in the first weeks of gestation correlate with a higher risk of miscarriage.[39–41] However, GDF-15 deficient mice do not show abnormalities in the embryonic development and are fully viable and fertile.[37] Soucek *et al*.[42] measured high levels of GDF-15 in seminal plasma from male donors irrespective of fertility status. Seminal GDF-15 does not appear to influence sperm cell viability or interact with vaginal or cervical cells, but it is capable of inhibiting the proliferation of peripheral blood mononuclear cells in a manner similar to TGF-β-1, but at higher effective concentrations. Moreover, GDF-15-induced the expression of FOXP3 in the CD4 + CD25 + peripheral blood mononuclear cells population from healthy donors.[42] Thus, the role of GDF-15 in human reproduction might comprise the meticulous regulation of the immune response during conception, implantation and early embryonic development.

Research showing that GDF-15 is linked to low body weight, nutritional disorders, cancer-associated cachexia and the metabolic response in cancer patients may be critical for clinical practice.[32,43–45] Similar effects were also observed in experimental animals.[46] Therefore, if GDF-15 contributes to complex stimulatory or inhibitory circuits for the regulation of adipose tissue homeostasis, novel therapeutic approaches for the management of unfavorable disease outcomes or therapeutic side effects may be offered. Johnen *et al*.[47] showed that GDF-15 can modulate both orexigenic and anorexigenic hypothalamic mediators and therefore indirectly suppress food intake. However, recent results show a direct role for GDF-15, as it was found to be expressed in different adipose tissue depots, and being regulated by, for example, leptin and IL1-β.[48] Expression of GDF-15 is elevated in patients with obesity comorbidities, suggesting a response to cellular stress or tissue damage.[45] Interestingly, Kim *et al*.[49] demonstrated that breast cancer cell line MDA-MB-231 responses to adipocyte-conditioned medium by dramatic increase of GDF-15 expression, resulting in enhanced invasivity of cancer cells.

The physiological role for GDF-15 in adipose tissue remains largely unknown, and further research in this field in needed.

In summary, these findings suggest a role for GDF-15 in the regulation of immune activity, particularly for regulating the inflammatory response and mediating tissue protection or regeneration.

REGULATION OF GDF-15 EXPRESSION

Transcriptional regulation of GDF-15 expression is complex and consists of several independent pathways that depend on tissue, cellular and signaling contexts. GDF-15 is differentially expressed in a variety of adult tissues, especially in reproductive or neural tissue. Moreover, GDF-15 is expressed in extraembryonal tissues, indicating some involvement in embryonic development.[39–41,50] This complexity in tissue distribution is reflected on the molecular level, implying the utilization of particular transcription machinery or tissue-specific upstream signaling. The GDF-15 promoter sequence is conserved in murine, rat and human tissues and consists of a TATA-like sequence as well as SP1 and AP1/2 consensus sites.[9] The promoter of GDF-15 contains two distinct p53-binding sites with different binding affinity to p53 *in vitro*. Interestingly, Wong *et al*.[51] identified a novel p53 transcriptional repressor element in close proximity of the p53-binding sites, suggesting a complex regulation of activation of GDF-15 in a manner dependent on cell or signaling context. GDF-15 represents a typical gene of the adaptive response to cellular stress, as its expression is generally low in quiescent cells, but is rapidly enhanced by different stress stimuli that employ different signaling pathways. Upon stimulation, GDF-15 is strongly expressed in response to NSAIDs and generally results in an antiproliferative phenotype. NSAIDs, used commonly to treat pain and inflammation, inhibit cyclooxygenase-1 and -2 and subsequently activate Egr-1 and p53 transcription factors to induce cell-cycle arrest.[52] GDF-15 was shown to be activated by NSAIDs through the p53 pathway[53] and is presumed to be one of the core mediators of NSAID-mediated cell-cycle arrest. However, the effects of NSAIDs on GDF-15 expression are not necessarily mediated by cyclooxygenase inhibition,[54] and other mechanisms have been proposed. GDF-15 expression-mediated cell growth arrest or apoptosis has been induced by various chemicals, particularly NSAIDs, and has often required p53/p21$^{Cip1/Waf1}$ activation.[53] More recent studies also demonstrated p53-independent activation of GDF-15, including GSK3β, C/EBP, ATF3[55] or Sp-1/Egr-1.[56] Lincova *et al*.[57] separated the mechanisms for GDF-15 induction by NSAIDs and cell growth arrest induced by cyclooxygenase-2 inhibition, suggesting GDF-15 transcriptional regulation independent of cell cycle or lipid signaling. The engagement of GDF-15 in antitumorigenic activities appears to be highly complex, depending on the structure and pharmacokinetic properties of particular NSAID or its metabolites. In APC/Min mice fed the NSAID sulindac either as a prodrug (DM-sulindac) or a pharmacologically active chemical with antitumor effects (sulindac sulfide), GDF-15 was induced in the liver parenchyma only with sulindac sulfide and not the prodrug.[58] Similarly, GDF-15 expression follows NSAID-induced apoptosis in oral cavity SCC1483 cancer cells, and conditioned medium containing GDF-15 potently inhibits proliferation of these cells.[59] The direct effects of GDF-15-mediated inhibition of cancer cell growth were also described in ovarian cancer cell lines SKOV3 and OVCAR3 that were treated with different NSAIDs.[60]

A number of plant-derived organic compounds promising novel anti-tumor effects were shown to induce GDF-15 expression that was preceded by p53 activation (e.g., organosulfuryl structures).[61] Heavy metals, DNA damaging agents, hypoxia or high cell density also stimulate GDF-15 expression in a p53-dependent manner.[62] In addition, particular saponins induce GDF-15 in a PI3K-dependent manner.[63] Further evidence that GDF-15 has a role in cellular stress responses was presented in the work of Schlittenhardt *et al*., which showed enhanced expression of GDF-15 induced by oxidized low-density lipoproteins, TNFα, certain ceramides or hydrogen peroxide. This group also demonstrated the immunohistochemical colocalization of GDF-15 with PARP, caspase-3, manganese, super-oxide dismutase, c-Jun and p53 in native atherosclerotic tissue.[64] Taken together, these findings suggest that GDF-15 can be induced by a broad spectrum of cellular or tissue events leading to activation of different intracellular pathways that result in complex phenotypes.

GDF-15 IN CANCER PROGRESSION, SYSTEMIC AND IMMUNE RESPONSE

GDF-15 is generally considered to be part of the cell's antitumorigenic actions, largely because its expression is crucial for the chemopreventive effects of various compounds.[57,58] However, elevated GDF-15 expression has often been reported during cancer progression, including gastric, ovarian, prostate or breast cancers (see Table 1) with various impact on tumors.[4,65,66] Despite that the GDF-15 expression profile has been well described in various cancers, its specific role in tumor development remains unclear (Figure 1). For example, in breast or gastric cancer, GDF-15 has been shown to be upregulated upon the activation of the MAPK-ERK1/2 or PKB/Akt pathways recruiting the SP-1 family of transcription factors.[67] GDF-15 also induces the phosphorylation and activation of ErbB receptors, mTOR/Akt and ERK1/2 pathways. A potential result of these signal integrations is HIF-1 and VEGF activation. Moreover, inhibition or specific downregulation of ErbB2 also inhibited GDF-15-mediated downstream signaling.[68] These findings indicate the importance of GDF-15 clinically, especially in ErbB2 (HER2)-positive cancers that are sensitive to small molecular inhibitors, such as lapatinib.[69] GDF-15 is strongly upregulated in hepatocellular carcinoma and other liver diseases, such as fibrosis or cirrhosis induced by hepatitis C virus.[70] GDF-15 autocrine signaling of transformed or infected hepatocytes then induces Akt, GSK-3/β catenin, Raf phosphorylation and other downstream targets, such as cell-cycle regulators (cyclins A2, E1 and D2) or adhesion molecules (E-cadherin). Interestingly, impairing GDF-15 can inhibit viral replication.[70] In malignant melanomas, GDF-15 is highly overexpressed,[71] and it is able to mimic VEGF in the neovascularization in the tumor site.[72] Similarly, in malignant glioblastomas, GDF-15 is upregulated as a reaction to anoxia, suggesting more general involvement in vascularization development.[73] Moreover, experimental decrease in GDF-15 expression clearly enhanced natural killer T-cell-mediated cytotoxicity, which increased the immunogenicity of glioma cells[74] similar to the effects of TGF-β downregulation.[75] Furthermore, GDF-15 depletion delays the growth of gliomas in mice *in vivo*. It is likely that GDF-15 acts as a potent suppressor of immune cells while simultaneously enhancing cancer cell growth through autocrine signaling. These observations emphasize the importance of assessing the role of the interactions within the tumor microenvironment for a context-dependent role of GDF-15. Interestingly, two antagonistic *in vivo* studies were published recently. Senapati *et al*.[76] demonstrated that ectopic overexpression of GDF-15 led to increased dissemination capacity of PCa cells. However, Zimmers *et al*.[58] showed that loss of GDF-15 expression abolished the chemopreventive effects of NSAIDs in animal models of hereditary colon cancer.

Thus, the primary effect of GDF-15 on cancer progression can be linked to the regulation of immune responses in the process of tissue regeneration. GDF-15 has been described as a negative regulator of macrophage activation by suppressing the release of TNF-α, IL-1, IL-2 and MCS-F, thus inhibiting the positive feedback of local inflammatory signaling similar to the effects of TGF-β.[3] However, the molecular mechanisms behind these immunosuppressive effects remain unclear despite several hypotheses that

Table 1. Clinical and biological aspects of GDF-15 expression in different cancer types

Cancer type	*Clinical prognostic information*	*Expression change and upstream regulation*	*Downstream signaling induced by GDF-15*	*Molecular and/or cellular phenotype induced by GDF-15*	*References*
Bladder	Candidate epigenetic biomarker	?	?	?	101
Breast	?	↑ via AKT ERK1/2–mTOR	ErbB2–AKT–ERK1/2–c-Src–p38–JNK	Enhanced invasion via c-Src	67,68,81,102
Colorectal	Association with tumor progression	↑ via p53	?	p53 dependent apoptosis	103,104
Gastric	Candidate biomarker	↑	EGFR(ErbB2)–MAPK1/2–ERK 1/2–Akt/mTOR	HIF-1α–VEGFA expression	65,68
Glioblastoma	Candidate biomarker	↑	?	Enhanced proliferation immune escape *in vivo*	74,105
Hepatocellular (HCV associated)	Candidate biomarker	↑	AKT–GSK-3β–c-Raf	Enhanced proliferation and invasion	70
Head and neck	Associated with radioresistant phenotype	↑	?	?	106
Melanoma	Association with tumor progression, metastases formation and vascular development	↑ via B-Raf	?	B-Raf–GDF-15 dependent vascularization	66,71,72
Oesophagus/gastric	Elevated; association with inflammation	↑	?	?	107
Ovarian	Prognostic biomarker	↑	?	?	50,108
Pancreas	Candidate biomarker	↑	?	?	109,110
Prostate	Prognostic biomarker	↑	PKB/Akt–FAK/RhoA	Reorganization of actin architecture enhanced motility metastatic development *in vivo*	57,76,79, 86,89,111

Abbreviations: FAK, focal adhesion kinases; GDF-15, growth/differentiation factor-15; JNK, c-Jun N-terminal kinase; mTOR, mammalian target of rapamycin. ↑ Indicates elevated expression, ? indicates unknown or so far insufficient data.

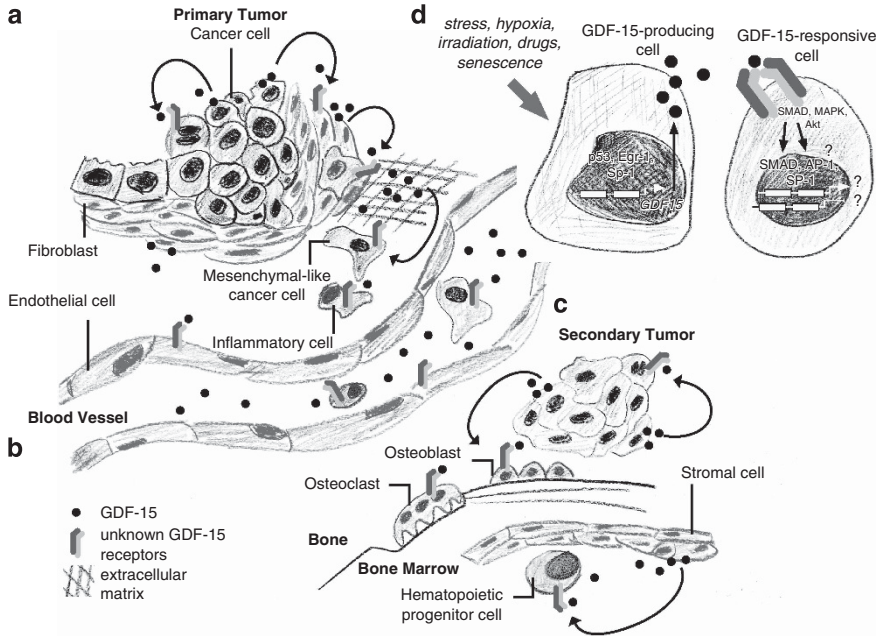

Figure 1. Schematic illustration of growth/differentiation factor-15 (GDF-15) action in tissue microenvironment and cancer progression. (**a**) GDF-15 is secreted by a primary tumor or released from extracellular matrix, affecting both the tumor and adjacent stromal or immune cells responsive to GDF-15. (**b**) GDF-15 is released to blood stream and contributing to tumor spreading, vascularization and immunosuppression. (**c**) GDF-15 is involved in remodeling of bone architecture by action on both osteoblasts and osteoclasts, affecting the bone-marrow microenvironment and stem-cell niche formation. (**d**) GDF-15 expression is induced upon various stimuli, for example, by p53 and/or Sp1-Egr-1 dependent transcription. GDF-15 induces signaling pathway comprising of so far identified SMAD, MAPK and Akt and activating transcription from SMAD, AP-1 and Sp-1 driven promoters.

focus on interactions with regulatory T lymphocytes in a context already defined for TGF-β in various cancers.[42,77] Taken together, the cancer-associated elevated expression of GDF-15 may have strong predictive potential that could justify the introduction of GDF-15 clinically as a biomarker for particular cancers. Moreover, focusing on its immunosuppressive characteristics, GDF-15 may be specifically targeted to restore the immune-mediated anti-tumor response.

GDF-15 IN PCA

GDF-15 as a biomarker

For PCa, there is a long-term need for both specific and robust markers that would allow precise prediction and estimation of disease outcome. At, present, only PSA was introduced to clinical practice. However, total levels of PSA in serum are not cancer specific, as they are found even in benign diseases, leading to potential false-positive diagnosis of PCa. Improvement was achieved by analyzing alternative molecular forms of PSA, which decreased the number of cancer-negative biopsies in indicated cases.[78] Of particular importance, the measurement of GDF-15 in serum was shown to increase the precision of the information potential of PSA and its forms. The presence of GDF-15 in serum is slightly decreased in BPH or localized PCa compared with normal prostate tissue,[79] but it is elevated in metastatic disease.[80,81] Interestingly, the inflammatory events in prostate tissue are considered to trigger the transition from normal to benign hyperplasic state and probably reflect an individual's sensitivity to autoimmune lesions.[82,83] Inflammatory changes of glandular architecture followed by increase of stromal tissue in BPH negatively correlate with GDF-15 expression.[84,85]

Despite the presence of several molecular forms of GDF-15, the analysis of total GDF-15 serum levels showed its clear discriminative capacity for PCa mortality and disease outcome, which justifies further prospective studies to potentially introduce GDF-15 as a clinically important biomarker for PCa.[86] As such, the analysis of overall GDF-15 may offer a robust screening method.

Role of GDF-15 in PCa development

Tumor development can be considered either as uncontrolled regeneration or as cellular reprogramming or reversion to the early developmental stages. Thus, lessons from embryonic development can shed light on the complex signaling in organogenesis and tissue formation. In the early development of the normal mouse prostate, GDF-15 is dynamically expressed in dividing epithelium originating from the urogenital sinus and buds, and its expression falls when the stage of developed prostate lobes has been reached.[87] GDF-15 is then reactivated during prostate maturation, and its expression correlates with differentiation markers (e.g., K19). Thus, data from embryonic, fetal and early postnatal murine development suggest a clear dual function for GDF-15 in the regulation of epithelial proliferation in the urogenital sinus as well as its differentiation in the later stages of prostate lobular structure formation.[87] A PCa model based on modified SV-40 region driven by the prostate-specific rat probasin promoter developed by Kasper *et al*.[88] (*CD-1-Tg(Pbsn-Tag) 12T10Rjm,* according to the Cancer Model Database) allowed for the detailed study of GDF-15 in the development of prostate intraepithelial neoplasias, which is considered comparable to human prostate intraepithelial neoplasias. Using this model, Noorali *et al*.[87] showed clear differences in GDF-15 expression among normally developing prostate, prostate hyperplasia and prostate intraepithelial neoplasias. Although the GDF-15 pattern of expression in normal tissue shows two clear peaks (epithelial proliferation in buds and lobes, differentiation of mature prostate), its expression is attenuated in the maturing prostate and is accompanied with a loss of differentiation markers in transgenic tissue showing progression from hyperplasia to prostate intraepithelial neoplasias. Furthermore, GDF-15 expression is strongly upregulated in the tumorigenic state that follows. Moreover, GDF-15 expression is enhanced in developed PCa similar to other cancer types.

Despite that there is low genetic variation in the GDF-15 coding sequence, and existing single nucleotide polymorphisms were not associated with PCa susceptibility,[89] the function of wild-type GDF-15 and its H6D variant can be discriminated in developed PCa. In athymic nude mice inoculated with DU145 PCa cells transfected with appropriate coding sequences, the H6D variant clearly interfered with tumor development by lowering levels of cyclin D1 and IGF-1 in the serum resulting in smaller tumors than in controls with wild-type GDF-15.[90] However, the systemic role for body-weight regulation and the induction of tumor associated cachexia is likely not compromised in the H6D variant compared with wild-type GDF-15, as both proteins significantly reduce the amount of abdominal fat in experimental mice and reduce adipose tissue signaling.[90]

A common problem associated with long-term PCa therapy is the development of hormone refractory PCa and resistance to chemotherapy. It is estimated that about 50% of patients treated by first-line castration do not respond to second-line of chemotherapy with Docetaxel.[91] The expression of GDF-15 in PC3 cells with acquired resistance to Docetaxel is increased after chemotherapy exposure compared with parental PC3s. Moreover, a similar trend has been observed in the serum/plasma of patients with Docetaxel-resistant PCa with a clear impact on patients' survival.[92] This correlates well with previously published data;[93] however, *in vitro* data showed a direct link between GDF-15 and Docetaxel resistance. Androgen independent PCa PC3 cells treated with GDF-15 became partially resistant to Docetaxel and Docetaxel-resistant PC3 cells treated with GDF-15 shRNA showed restored susceptibility to Docetaxel.[92]

In androgen-sensitive LNCaP cells, GDF-15 is expressed and supports proliferation and clonogenic cell growth. GDF-15 silencing in the LN3 subline of LNCaP cells, which are characterized by a high-metastatic potential, decreased proliferation rate and reduced anchor-independent cell growth on soft agar.[94] In PC3 and DU145 cells, GDF-15 is virtually unexpressed;[95] however, these cells retain sensitivity to GDF-15 under particular conditions. The p53-negative PC3 cells responded to GDF-15 treatment by reducing mobility through matrigel columns.[96] Similarly, for DU145, a slight tumor suppressive effect was reported *in vivo*.[90]

To identify a systemic role of GDF-15 in PCa bone metastasis, Wakchoure *et al*.[46] inoculated athymic nude mice with DU-145 PCa cells overexpressing GDF-15. The histomorphological and X-ray analyses showed enhanced osteoblast differentiation and bone-remodeling activity in sites of bone metastases. This study also showed enhanced osteoclast numbers in metastasis sites; however, using an *in vitro* macrophage model, another study demonstrated clear inhibitory effects of GDF-15 on osteoclast formation. Under experimental *in vitro* conditions, GDF-15 inhibited MCSF-RANKL-induced osteoclast differentiation that were derived either from the macrophage cell line RAW264.7 or mononuclear precursors isolated from murine bone marrow. Impaired differentiation resulted in reduced osteoclast numbers in culture and decreased bone resorption. On the molecular level, GDF-15 induced the retention of IκB, an inhibitor of the NFκB transcription factor, in the cytoplasm, thus preventing NFκB-mediated expression of the key transcription factor c-*fos* and the osteoclast hallmark enzymes cathepsin K and carbonic anhydrase II.[97] Interestingly, GDF-15 is upregulated by vitamin D3 ($1,25(OH)_2D_3$) in the androgen-dependent PCa cells LNCaP.[98] The GDF-15 protein produced by $1,25(OH)_2D_3$-stimulated LNCaP cells was the biologically active form that interferes with MCSF-RANKL signaling independently of osteoprotegerin, a physiological

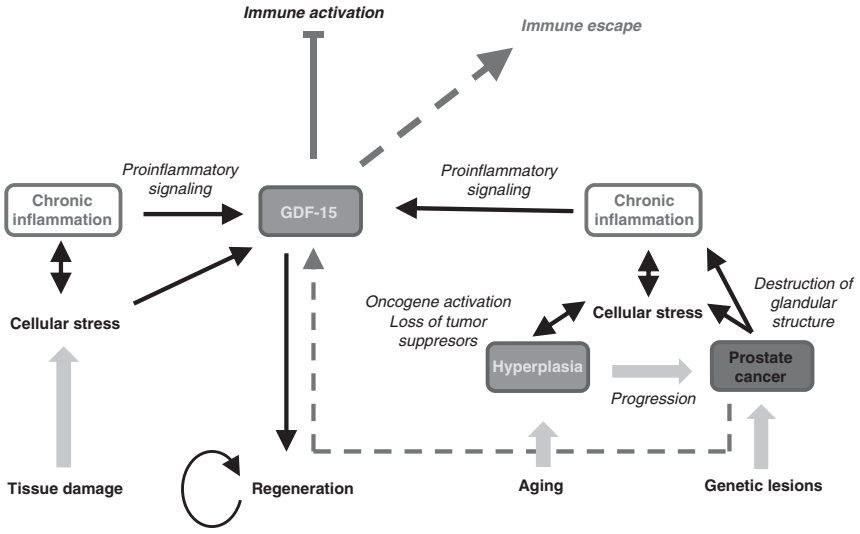

Figure 2. Proposed role of growth/differentiation factor-15 (GDF-15) in prostate cancer (PCa) progression. GDF-15 is induced by cellular stress upon tissue damage or inflammation and physiologically protects the lesion site from the inadequate reaction of immune system. Under pathological conditions, such as developed PCa, GDF-15 is induced as a result of cellular stress caused by changes and/or destruction of prostate tissue architecture, therefore inducing immunoprotective status of cancer-lesioned site.

regulator of osteoclast differentiation and activity.[97] Further evidences for a role for GDF-15 in cancer spreading and distant metastasis formation were reported by Senapati *et al*.[76] This *in vitro* study described enhanced motility and invasion capabilities and changes in the actin cytoskeletal architecture of PC3 and LNCaP cells overexpressing GDF-15. The induction of intracellular signaling by GDF-15 led to the activation of focal adhesion kinases and the small GTPase RhoA, suggesting that GDF-15 moderates direct control over architectural rearrangements and subsequent cell motility. However, using ovarian and prostate SKOV-3 and PC3 cells, respectively, Cheng *et al*.[96] showed that GDF-15 inhibited cellular migration through matrigel columns through a p53-dependent mechanism. Similarly, GDF-15 has been described as a mediator of NSAIDs-induced inhibition of migration of PCa cells.[99] According to these experimental data, GDF-15 effects may vary depending on signaling status, the genetic background of target cell populations, particularly on the presence of the androgen receptor and/or p53 activity and interaction with immune system (Figure 2). Contextual pleiotropy and dual role in cancer, which is general characteristic of TGF-β family cytokines[100] is most likely characteristic also of GDF-15. However, detail mechanisms of its both tumor suppressor and/or promoter action needs to be revealed.

CONCLUSIONS

GDF-15 is a distant member of the TGF-β family and is strongly expressed in a great variety of human cancers including PCa; however, its role in cancer pathophysiology remains ambiguous. Nevertheless, the link between GDF-15 expression and the tumor stage or disease outcome is informative, suggesting that GDF-15 may be a clinically relevant biomarker for particular cancers. The physiological role of GDF-15 may involve mediating interactions between different cellular populations and the immune system or enabling mutual regulation in certain microenvironments. Thus, the suppression of certain immune cell populations may be the core systemic mechanism for the role of GDF-15 in cancer development and progression. GDF-15 was first recognized as a factor interfering with macrophage activation. Later studies showed its inhibitory role on the effects of NSAIDs and the suppression of macrophage-derived osteoclasts or regulatory T lymphocytes. The role of GDF-15 in embryonic development remains unresolved. In mice, GDF-15 is not necessary for proper development and knockouts are fully viable and fertile; however, in humans, it likely has a role in feto-maternal interactions and may prevent immune rejection *in utero*. There is increasing evidence for the involvement of GDF-15 in tissue regeneration or the reaction to different stress conditions. GDF-15-mediated suppressive effects often mimic those observed by generic TGF-βs, but there are differences in target population responses. Of particular importance is to clarify the intracellular signals ranging from the receptor formation to the interacting partners that mediate the effects of GDF-15. Increased knowledge of GDF-15-induced signaling pathways in either producing or receptive cells will contribute to the understanding of events that form the complex communication network within the tumor microenvironment. The nature of GDF-15 proteins has two sides, with both tumor-suppressor and oncogenic characteristics. As the cellular, tissue and systemic effects of GDF-15 signaling in well-defined experimental conditions has shown, it is likely that GDF15 is an active and important player in the development of PCa rather than a stress-induced bystander. Therefore, introducing GDF-15 into clinical discussions may offer new possibilities to better understand cancer development and potentially enhance diagnostic or therapeutic strategies.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported by grants IGA MZD 9600-4/2008 and IGA MZD 9956-4/2008, Grant number MSM0021622430 from the Ministry of Education, Youth and Sports of the Czech Republic and by the project FNUSA-ICRC (no. CZ.1.05/1.1.00/02.0123) from the European Regional Development Fund.

REFERENCES

1 Dranoff G. Cytokines in cancer pathogenesis and cancer therapy. *Nat Rev Cancer* 2004; **4**: 11 - 22.
2 Yang L, Moses HL. Transforming growth factor beta: tumor suppressor or promoter? Are host immune cells the answer? *Cancer Res* 2008; **68**: 9107 - 9111.

3 Bootcov MR, Bauskin AR, Valenzuela SM, Moore AG, Bansal M, He XY *et al.* MIC-1, a novel macrophage inhibitory cytokine, is a divergent member of the TGF-beta superfamily. *Proc Natl Acad Sci USA* 1997; **94**: 11514 - 11519.
4 Bauskin AR, Brown DA, Kuffner T, Johnen H, Luo XW, Hunter M *et al.* Role of macrophage inhibitory cytokine-1 in tumorigenesis and diagnosis of cancer. *Cancer Res* 2006; **66**: 4983 - 4986.
5 Baek SJ, Kim KS, Nixon JB, Wilson LC, Eling TE. Cyclooxygenase inhibitors regulate the expression of a TGF-beta superfamily member that has proapoptotic and antitumorigenic activities. *Mol Pharmacol* 2001; **59**: 901 - 908.
6 Fairlie WD, Moore AG, Bauskin AR, Russell PK, Zhang HP, Breit SN. MIC-1 is a novel TGF-beta superfamily cytokine associated with macrophage activation. *J Leukoc Biol* 1999; **65**: 2 - 5.
7 Hromas R, Hufford M, Sutton J, Xu D, Li Y, Lu L. PLAB, a novel placental bone morphogenetic protein. *Biochim Biophys Acta* 1997; **1354**: 40 - 44.
8 Yokoyama-Kobayashi M, Saeki M, Sekine S, Kato S. Human cDNA encoding a novel TGF-beta superfamily protein highly expressed in placenta. *J Biochem* 1997; **122**: 622 - 626.
9 Bottner M, Laaff M, Schechinger B, Rappold G, Unsicker K, Suter-Crazzolara C. Characterization of the rat, mouse, and human genes of growth/differentiation factor-15/macrophage inhibiting cytokine-1 (GDF-15/MIC-1). *Gene* 1999; **237**: 105 - 111.
10 Brown DA, Bauskin AR, Fairlie WD, Smith MD, Liu T, Xu N *et al.* Antibody-based approach to high-volume genotyping for MIC-1 polymorphism. *Biotechniques* 2002; **33**: 118 - 120, 122, 124 passim.
11 Hayes VM, Severi G, Southey MC, Padilla EJ, English DR, Hopper JL *et al.* Macrophage inhibitory cytokine-1 H6D polymorphism, prostate cancer risk, and survival. *Cancer Epidemiol Biomarkers Prev* 2006; **15**: 1223 - 1225.
12 Lindmark F, Zheng SL, Wiklund F, Bensen J, Balter KA, Chang B *et al.* H6D polymorphism in macrophage-inhibitory cytokine-1 gene associated with prostate cancer. *J Natl Cancer Inst* 2004; **96**: 1248 - 1254.
13 Bauskin AR, Zhang HP, Fairlie WD, He XY, Russell PK, Moore AG *et al.* The propeptide of macrophage inhibitory cytokine (MIC-1), a TGF-beta superfamily member, acts as a quality control determinant for correctly folded MIC-1. *EMBO J* 2000; **19**: 2212 - 2220.
14 Brunner AM, Lioubin MN, Marquardt H, Malacko AR, Wang WC, Shapiro RA *et al.* Site-directed mutagenesis of glycosylation sites in the transforming growth factor-beta 1 (TGF beta 1) and TGF beta 2 (414) precursors and of cysteine residues within mature TGF beta 1: effects on secretion and bioactivity. *Mol Endocrinol* 1992; **6**: 1691 - 1700.
15 Bauskin AR, Brown DA, Junankar S, Rasiah KK, Eggleton S, Hunter M *et al.* The propeptide mediates formation of stromal stores of PROMIC-1: role in determining prostate cancer outcome. *Cancer Res* 2005; **65**: 2330 - 2336.
16 Abd El-Aziz SH, Endo Y, Miyamaori H, Takino T, Sato H. Cleavage of growth differentiation factor 15 (GDF15) by membrane type 1-matrix metalloproteinase abrogates GDF15-mediated suppression of tumor cell growth. *Cancer Sci* 2007; **98**: 1330 - 1335.
17 Anand IS, Kempf T, Rector TS, Tapken H, Allhoff T, Jantzen F *et al.* Serial measurement of growth-differentiation factor-15 in heart failure: relation to disease severity and prognosis in the Valsartan Heart Failure Trial. *Circulation* 2010; **122**: 1387 - 1395.
18 Daniels LB, Clopton P, Laughlin GA, Maisel AS, Barrett-Connor E. Growth-differentiation factor-15 is a robust, independent predictor of 11-year mortality risk in community-dwelling older adults: the Rancho Bernardo study. *Circulation* 2011; **123**: 2101 - 2110.
19 Kempf T, Horn-Wichmann R, Brabant G, Peter T, Allhoff T, Klein G *et al.* Circulating concentrations of growth-differentiation factor 15 in apparently healthy elderly individuals and patients with chronic heart failure as assessed by a new immunoradiometric sandwich assay. *Clin Chem* 2007; **53**: 284 - 291.
20 Kempf T, von Haehling S, Peter T, Allhoff T, Cicoira M, Doehner W *et al.* Prognostic utility of growth differentiation factor-15 in patients with chronic heart failure. *J Am Coll Cardiol* 2007; **50**: 1054 - 1060.
21 Kempf T, Wollert KC. Growth-differentiation factor-15 in heart failure. *Heart Fail Clin* 2009; **5**: 537 - 547.
22 Wang F, Guo Y, Yu H, Zheng L, Mi L, Gao W. Growth differentiation factor 15 in different stages of heart failure: potential screening implications. *Biomarkers* 2010; **15**: 671 - 676.
23 Wiklund FE, Bennet AM, Magnusson PK, Eriksson UK, Lindmark F, Wu L *et al.* Macrophage inhibitory cytokine-1 (MIC-1/GDF15): a new marker of all-cause mortality. *Aging Cell* 2010; **9**: 1057 - 1064.
24 Dinh W, Futh R, Lankisch M, Hess G, Zdunek D, Scheffold T *et al.* Growth-differentiation factor-15: a novel biomarker in patients with diastolic dysfunction? *Arq Bras Cardiol* 2011; **97**: 65 - 75.
25 Stahrenberg R, Edelmann F, Mende M, Kockskamper A, Dungen HD, Luers C *et al.* The novel biomarker growth differentiation factor 15 in heart failure with normal ejection fraction. *Eur J Heart Fail* 2010; **12**: 1309 - 1316.
26 Kempf T, Zarbock A, Widera C, Butz S, Stadtmann A, Rossaint J *et al.* GDF-15 is an inhibitor of leukocyte integrin activation required for survival after myocardial infarction in mice. *Nat Med* 2011; **17**: 581 - 588.
27 de Jager SC, Bermudez B, Bot I, Koenen RR, Bot M, Kavelaars A *et al.* Growth differentiation factor 15 deficiency protects against atherosclerosis by attenuating CCR2-mediated macrophage chemotaxis. *J Exp Med* 2011; **208**: 217 - 225.
28 Song H, Yin D, Liu Z. GDF-15 promotes angiogenesis through modulating p53/HIF-1α signaling pathway in hypoxic human umbilical vein endothelial cells. *Mol Biol Rep* 2011; e-pub ahead of print 20 July 2011.
29 Brown DA, Moore J, Johnen H, Smeets TJ, Bauskin AR, Kuffner T *et al.* Serum macrophage inhibitory cytokine 1 in rheumatoid arthritis: a potential marker of erosive joint destruction. *Arthritis Rheum* 2007; **56**: 753 - 764.
30 Tamary H, Shalev H, Perez-Avraham G, Zoldan M, Levi I, Swinkels DW *et al.* Elevated growth differentiation factor 15 expression in patients with congenital dyserythropoietic anemia type I. *Blood* 2008; **112**: 5241 - 5244.
31 Theurl I, Finkenstedt A, Schroll A, Nairz M, Sonnweber T, Bellmann-Weiler R *et al.* Growth differentiation factor 15 in anaemia of chronic disease, iron deficiency anaemia and mixed type anaemia. *Br J Haematol* 2009; **148**: 449 - 455.
32 Dostalova I, Roubicek T, Bartlova M, Mraz M, Lacinova Z, Haluzikova D *et al.* Increased serum concentrations of macrophage inhibitory cytokine-1 in patients with obesity and type 2 diabetes mellitus: the influence of very low calorie diet. *Eur J Endocrinol* 2009; **161**: 397 - 404.
33 Sugulle M, Dechend R, Herse F, Weedon-Fekjaer MS, Johnsen GM, Brosnihan KB *et al.* Circulating and placental growth-differentiation factor 15 in preeclampsia and in pregnancy complicated by diabetes mellitus. *Hypertension* 2009; **54**: 106 - 112.
34 Tanno T, Noel P, Miller JL. Growth differentiation factor 15 in erythroid health and disease. *Curr Opin Hematol* 2011; **17**: 184 - 190.
35 Zimmers TA, Jin X, Hsiao EC, McGrath SA, Esquela AF, Koniaris LG. Growth differentiation factor-15/macrophage inhibitory cytokine-1 induction after kidney and lung injury. *Shock* 2005; **23**: 543 - 548.
36 Duong Van Huyen JP, Cheval L, Bloch-Faure M, Belair MF, Heudes D, Bruneval P *et al.* GDF15 triggers homeostatic proliferation of acid-secreting collecting duct cells. *J Am Soc Nephrol* 2008; **19**: 1965 - 1974.
37 Strelau J, Strzelczyk A, Rusu P, Bendner G, Wiese S, Diella F *et al.* Progressive postnatal motoneuron loss in mice lacking GDF-15. *J Neurosci* 2009; **29**: 13640 - 13648.
38 Schindowski K, von Bohlen und Halbach O, Strelau J, Ridder DA, Herrmann O, Schober A *et al.* Regulation of GDF-15, a distant TGF-beta superfamily member, in a mouse model of cerebral ischemia. *Cell Tissue Res* 2011; **343**: 399 - 409.
39 Marjono AB, Brown DA, Horton KE, Wallace EM, Breit SN, Manuelpillai U. Macrophage inhibitory cytokine-1 in gestational tissues and maternal serum in normal and pre-eclamptic pregnancy. *Placenta* 2003; **24**: 100 - 106.
40 Tong S, Marjono B, Brown DA, Mulvey S, Breit SN, Manuelpillai U *et al.* Serum concentrations of macrophage inhibitory cytokine 1 (MIC 1) as a predictor of miscarriage. *Lancet* 2004; **363**: 129 - 130.
41 Wallace EM, Marjono B, Brown DA, Crossley J, Tong S, Aitken D *et al.* Maternal serum and amniotic fluid levels of macrophage inhibitory cytokine 1 in Down syndrome and chromosomally normal pregnancies. *Prenat Diagn* 2004; **24**: 224 - 226.
42 Soucek K, Slabakova E, Ovesna P, Malenovska A, Kozubik A, Hampl A. Growth/differentiation factor-15 is an abundant cytokine in human seminal plasma. *Hum Reprod* 2010; **25**: 2962 - 2971.
43 Dostalova I, Kavalkova P, Papezova H, Domluvilova D, Zikan V, Haluzik M. Association of macrophage inhibitory cytokine-1 with nutritional status, body composition and bone mineral density in patients with anorexia nervosa: the influence of partial realimentation. *Nutr Metab (Lond)* 2010; **7**: 34.
44 Jatoi A. Weight loss in patients with advanced cancer: effects, causes, and potential management. *Curr Opin Support Palliat Care* 2008; **2**: 45 - 48.
45 Vila G, Riedl M, Anderwald C, Resl M, Handisurya A, Clodi M *et al.* The relationship between insulin resistance and the cardiovascular biomarker growth differentiation factor-15 in obese patients. *Clin Chem* 2011; **57**: 309 - 316.
46 Wakchoure S, Swain TM, Hentunen TA, Bauskin AR, Brown DA, Breit SN *et al.* Expression of macrophage inhibitory cytokine-1 in prostate cancer bone metastases induces osteoclast activation and weight loss. *Prostate* 2009; **69**: 652 - 661.
47 Johnen H, Lin S, Kuffner T, Brown DA, Tsai VW, Bauskin AR *et al.* Tumor-induced anorexia and weight loss are mediated by the TGF-beta superfamily cytokine MIC-1. *Nat Med* 2007; **13**: 1333 - 1340.
48 Ding Q, Mracek T, Gonzalez-Muniesa P, Kos K, Wilding J, Trayhurn P *et al.* Identification of macrophage inhibitory cytokine-1 in adipose tissue and its secretion as an adipokine by human adipocytes. *Endocrinology* 2009; **150**: 1688 - 1696.

49 Kim JH, Kim KY, Jeon JH, Lee SH, Hwang JE, Lee JH *et al*. Adipocyte culture medium stimulates production of macrophage inhibitory cytokine 1 in MDA-MB-231 cells. *Cancer Lett* 2008; **261**: 253 - 262.
50 Bock AJ, Stavnes HT, Kempf T, Trope CG, Berner A, Davidson B *et al*. Expression and clinical role of growth differentiation factor-15 in ovarian carcinoma effusions. *Int J Gynecol Cancer* 2010; **20**: 1448 - 1455.
51 Wong J, Li PX, Klamut HJ. A novel p53 transcriptional repressor element (p53TRE) and the asymmetrical contribution of two p53 binding sites modulate the response of the placental transforming growth factor-beta promoter to p53. *J Biol Chem* 2002; **277**: 26699 - 26707.
52 Baek SJ, Kim JS, Moore SM, Lee SH, Martinez J, Eling TE. Cyclooxygenase inhibitors induce the expression of the tumor suppressor gene EGR-1, which results in the up-regulation of NAG-1, an antitumorigenic protein. *Mol Pharmacol* 2005; **67**: 356 - 364.
53 Yang H, Filipovic Z, Brown D, Breit SN, Vassilev LT. Macrophage inhibitory cytokine-1: a novel biomarker for p53 pathway activation. *Mol Cancer Ther* 2003; **2**: 1023 - 1029.
54 Baek SJ, Wilson LC, Lee CH, Eling TE. Dual function of nonsteroidal anti-inflammatory drugs (NSAIDs): inhibition of cyclooxygenase and induction of NSAID-activated gene. *J Pharmacol Exp Ther* 2002; **301**: 1126 - 1131.
55 Lee SH, Krisanapun C, Baek SJ. NSAID-activated gene-1 as a molecular target for capsaicin-induced apoptosis through a novel molecular mechanism involving GSK3beta, C/EBPbeta and ATF3. *Carcinogenesis* 2010; **31**: 719 - 728.
56 Yoshioka H, Kamitani H, Watanabe T, Eling TE. Nonsteroidal anti-inflammatory drug-activated gene (NAG-1/GDF15) expression is increased by the histone deacetylase inhibitor trichostatin A. *J Biol Chem* 2008; **283**: 33129 - 33137.
57 Lincova E, Hampl A, Pernicova Z, Starsichova A, Krcmar P, Machala M *et al*. Multiple defects in negative regulation of the PKB/Akt pathway sensitise human cancer cells to the antiproliferative effect of non-steroidal anti-inflammatory drugs. *Biochem Pharmacol* 2009; **78**: 561 - 572.
58 Zimmers TA, Gutierrez JC, Koniaris LG. Loss of GDF-15 abolishes sulindac chemoprevention in the ApcMin/+ mouse model of intestinal cancer. *J Cancer Res Clin Oncol* 2010; **136**: 571 - 576.
59 Kim KS, Rhee KH, Yoon JH, Lee JG, Lee JH, Yoo JB. Ginkgo biloba extract (EGb 761) induces apoptosis by the activation of caspase-3 in oral cavity cancer cells. *Oral Oncol* 2005; **41**: 383 - 389.
60 Kim JS, Baek SJ, Sali T, Eling TE. The conventional nonsteroidal anti-inflammatory drug sulindac sulfide arrests ovarian cancer cell growth via the expression of NAG-1/MIC-1/GDF-15. *Mol Cancer Ther* 2005; **4**: 487 - 493.
61 Bottone Jr FG, Baek SJ, Nixon JB, Eling TE. Diallyl disulfide (DADS) induces the antitumorigenic NSAID-activated gene (NAG-1) by a p53-dependent mechanism in human colorectal HCT 116 cells. *J Nutr* 2002; **132**: 773 - 778.
62 Kelly JA, Lucia MS, Lambert JR. p53 controls prostate-derived factor/macrophage inhibitory cytokine/NSAID-activated gene expression in response to cell density, DNA damage and hypoxia through diverse mechanisms. *Cancer Lett* 2009; **277**: 38 - 47.
63 Auyeung KK, Cho CH, Ko JK. A novel anticancer effect of astragalus saponins: Transcriptional activation of NSAID-activated gene. *Int J Cancer* 2009; **125**: 1082 - 1091.
64 Schlittenhardt D, Schober A, Strelau J, Bonaterra GA, Schmiedt W, Unsicker K *et al*. Involvement of growth differentiation factor-15/macrophage inhibitory cytokine-1 (GDF-15/MIC-1) in oxLDL-induced apoptosis of human macrophages *in vitro* and in arteriosclerotic lesions. *Cell Tissue Res* 2004; **318**: 325 - 333.
65 Baek KE, Yoon SR, Kim JT, Kim KS, Kang SH, Yang Y *et al*. Upregulation and secretion of macrophage inhibitory cytokine-1 (MIC-1) in gastric cancers. *Clin Chim Acta* 2009; **401**: 128 - 133.
66 Riker AI, Enkemann SA, Fodstad O, Liu S, Ren S, Morris C *et al*. The gene expression profiles of primary and metastatic melanoma yields a transition point of tumor progression and metastasis. *BMC Med Genomics* 2008; **1**: 13.
67 Wollmann W, Goodman ML, Bhat-Nakshatri P, Kishimoto H, Goulet Jr RJ, Mehrotra S *et al*. The macrophage inhibitory cytokine integrates AKT/PKB and MAP kinase signaling pathways in breast cancer cells. *Carcinogenesis* 2005; **26**: 900 - 907.
68 Kim KK, Lee JJ, Yang Y, You KH, Lee JH. Macrophage inhibitory cytokine-1 activates AKT and ERK-1/2 via the transactivation of ErbB2 in human breast and gastric cancer cells. *Carcinogenesis* 2008; **29**: 704 - 712.
69 Burris 3rd HA. Dual kinase inhibition in the treatment of breast cancer: initial experience with the EGFR/ErbB-2 inhibitor lapatinib. *Oncologist* 2004; **9** (Suppl 3): 10 - 15.
70 Si Y, Liu X, Cheng M, Wang M, Gong Q, Yang Y *et al*. Growth differentiation factor 15 is induced by hepatitis C virus infection and regulates hepatocellular carcinoma-related genes. *PLoS One* 2011; **6**: e19967.
71 Boyle GM, Pedley J, Martyn AC, Banducci KJ, Strutton GM, Brown DA *et al*. Macrophage inhibitory cytokine-1 is overexpressed in malignant melanoma and is associated with tumorigenicity. *J Invest Dermatol* 2009; **129**: 383 - 391.
72 Huh SJ, Chung CY, Sharma A, Robertson GP. Macrophage inhibitory cytokine-1 regulates melanoma vascular development. *Am J Pathol* 2010; **176**: 2948 - 2957.
73 Albertoni M, Shaw PH, Nozaki M, Godard S, Tenan M, Hamou MF *et al*. Anoxia induces macrophage inhibitory cytokine-1 (MIC-1) in glioblastoma cells independently of p53 and HIF-1. *Oncogene* 2002; **21**: 4212 - 4219.
74 Roth P, Junker M, Tritschler I, Mittelbronn M, Dombrowski Y, Breit SN *et al*. GDF-15 contributes to proliferation and immune escape of malignant gliomas. *Clin Cancer Res* 2010; **16**: 3851 - 3859.
75 Friese MA, Wischhusen J, Wick W, Weiler M, Eisele G, Steinle A *et al*. RNA interference targeting transforming growth factor-beta enhances NKG2D-mediated antiglioma immune response, inhibits glioma cell migration and invasiveness, and abrogates tumorigenicity *in vivo*. *Cancer Res* 2004; **64**: 7596 - 7603.
76 Senapati S, Rachagani S, Chaudhary K, Johansson SL, Singh RK, Batra SK. Overexpression of macrophage inhibitory cytokine-1 induces metastasis of human prostate cancer cells through the FAK-RhoA signaling pathway. *Oncogene* 2010; **29**: 1293 - 1302.
77 Ostroukhova M, Qi Z, Oriss TB, Dixon-McCarthy B, Ray P, Ray A. Treg-mediated immunosuppression involves activation of the Notch-HES1 axis by membrane-bound TGF-beta. *J Clin Invest* 2006; **116**: 996 - 1004.
78 Catalona WJ, Partin AW, Slawin KM, Brawer MK, Flanigan RC, Patel A *et al*. Use of the percentage of free prostate-specific antigen to enhance differentiation of prostate cancer from benign prostatic disease: a prospective multicenter clinical trial. *JAMA* 1998; **279**: 1542 - 1547.
79 Brown DA, Stephan C, Ward RL, Law M, Hunter M, Bauskin AR *et al*. Measurement of serum levels of macrophage inhibitory cytokine 1 combined with prostate-specific antigen improves prostate cancer diagnosis. *Clin Cancer Res* 2006; **12**: 89 - 96.
80 Nakamura T, Scorilas A, Stephan C, Yousef GM, Kristiansen G, Jung K *et al*. Quantitative analysis of macrophage inhibitory cytokine-1 (MIC-1) gene expression in human prostatic tissues. *Br J Cancer* 2003; **88**: 1101 - 1104.
81 Welsh JB, Sapinoso LM, Kern SG, Brown DA, Liu T, Bauskin AR *et al*. Large-scale delineation of secreted protein biomarkers overexpressed in cancer tissue and serum. *Proc Natl Acad Sci USA* 2003; **100**: 3410 - 3415.
82 Kramer G, Marberger M. Could inflammation be a key component in the progression of benign prostatic hyperplasia? *Curr Opin Urol* 2006; **16**: 25 - 29.
83 Kramer G, Mitteregger D, Marberger M. Is benign prostatic hyperplasia (BPH) an immune inflammatory disease? *Eur Urol* 2007; **51**: 1202 - 1216.
84 Taniguchi S, Taoka R, Inui M, Sugimoto M, Kakehi Y. Influence of inflammation and aging on macrophage inhibitory cytokine-1 gene expression in rat ventral prostate. *Urology* 2009; **73**: 410 - 414.
85 Taoka R, Tsukuda F, Ishikawa M, Haba R, Kakehi Y. Association of prostatic inflammation with down-regulation of macrophage inhibitory cytokine-1 gene in symptomatic benign prostatic hyperplasia. *J Urol* 2004; **171** (6 Pt 1): 2330 - 2335.
86 Brown DA, Lindmark F, Stattin P, Balter K, Adami HO, Zheng SL *et al*. Macrophage inhibitory cytokine 1: a new prognostic marker in prostate cancer. *Clin Cancer Res* 2009; **15**: 6658 - 6664.
87 Noorali S, Kurita T, Woolcock B, de Algara TR, Lo M, Paralkar V *et al*. Dynamics of expression of growth differentiation factor 15 in normal and PIN development in the mouse. *Differentiation* 2007; **75**: 325 - 336.
88 Kasper S, Sheppard PC, Yan Y, Pettigrew N, Borowsky AD, Prins GS *et al*. Development, progression, and androgen-dependence of prostate tumors in probasin-large T antigen transgenic mice: a model for prostate cancer. *Lab Invest* 1998; **78**: i - xv.
89 Cheng I, Krumroy LM, Plummer SJ, Casey G, Witte JS. MIC1 and IL1RN genetic variation and advanced prostate cancer risk. *Cancer Epidemiol Biomarkers Prev* 2007; **16**: 1309 - 1311.
90 Wang X, Chrysovergis K, Bienstock RJ, Shim M, Eling TE. The H6D variant of NAG-1/GDF15 inhibits prostate xenograft growth *in vivo*. *Prostate* 2011; e-pub ahead of print 1 August 2011.
91 Galsky MD, Vogelzang NJ. Docetaxel-based combination therapy for castration-resistant prostate cancer. *Annals Oncol* 2010; **21**: 2135 - 2144.
92 Zhao L, Lee BY, Brown DA, Molloy MP, Marx GM, Pavlakis N *et al*. Identification of candidate biomarkers of therapeutic response to docetaxel by proteomic profiling. *Cancer Res* 2009; **69**: 7696 - 7703.
93 Huang CY, Beer TM, Higano CS, True LD, Vessella R, Lange PH *et al*. Molecular alterations in prostate carcinomas that associate with *in vivo* exposure to chemotherapy: identification of a cytoprotective mechanism involving growth differentiation factor 15. *Clin Cancer Res* 2007; **13**: 5825 - 5833.
94 Chen S-J, Karan D, Johansson SL, Lin F-F, Zeckser J, Singh AP *et al*. Prostate-derived factor as a paracrine and autocrine factor for the proliferation

of androgen receptor-positive human prostate cancer cells. *Prostate* 2007; **67**: 557 - 571.

95 Karan D, Chen S-J, Johansson SL, Singh AP, Paralkar VM, Lin M-F *et al*. Dysregulated expression of MIC-1/PDF in human prostate tumor cells. *Biochem and Biophys Res Commun* 2003; **305**: 598 - 604.

96 Cheng JC, Chang HM, Leung PC. Wild-Type p53 attenuates cancer cell motility by inducing growth differentiation factor-15 expression. *Endocrinology* 2011; **152**: 2987 - 2995.

97 Vanhara P, Lincova E, Kozubik A, Jurdic P, Soucek K, Smarda J. Growth/differentiation factor-15 inhibits differentiation into osteoclasts--a novel factor involved in control of osteoclast differentiation. *Differentiation* 2009; **78**: 213 - 222.

98 Lambert JR, Kelly JA, Shim M, Huffer WE, Nordeen SK, Baek SJ *et al*. Prostate derived factor in human prostate cancer cells: gene induction by vitamin D via a p53-dependent mechanism and inhibition of prostate cancer cell growth. *J Cell Physiol* 2006; **208**: 566 - 574.

99 Wynne S, Djakiew D. NSAID inhibition of prostate cancer cell migration is mediated by Nag-1 induction via the p38 MAPK-p75(NTR) pathway. *Mol Cancer Res* 2010; **8**: 1656 - 1664.

100 Massague J. TGFbeta in Cancer. *Cell* 2008; **134**: 215 - 230.

101 Costa VL, Henrique R, Danielsen SA, Duarte-Pereira S, Eknaes M, Skotheim RI *et al*. Three epigenetic biomarkers, GDF15, TMEFF2, and VIM, accurately predict bladder cancer from DNA-based analyses of urine samples. *Clin Cancer Res* 2010; **16**: 5842 - 5851.

102 Park YJ, Lee H, Lee JH. Macrophage inhibitory cytokine-1 transactivates ErbB family receptors via the activation of Src in SK-BR-3 human breast cancer cells. *BMB Rep* **43**: 91 - 96.

103 Brown DA, Ward RL, Buckhaults P, Liu T, Romans KE, Hawkins NJ *et al*. MIC-1 serum level and genotype: associations with progress and prognosis of colorectal carcinoma. *Clin Cancer Res* 2003; **9**: 2642 - 2650.

104 Kim IY, Park SY, Kang Y, Thapa D, Choi HG, Kim JA. Role of nonsteroidal anti-inflammatory drug-activated gene-1 in docetaxel-induced cell death of human colorectal cancer cells with different p53 status. *Arch Pharm Res* 2011; **34**: 323 - 330.

105 Shnaper S, Desbaillets I, Brown DA, Murat A, Migliavacca E, Schluep M *et al*. Elevated levels of MIC-1/GDF15 in the cerebrospinal fluid of patients are associated with glioblastoma and worse outcome. *Int J Cancer* 2009; **125**: 2624 - 2630.

106 Lin TY, Chang JT, Wang HM, Chan SH, Chiu CC, Lin CY *et al*. Proteomics of the radioresistant phenotype in head-and-neck cancer: Gp96 as a novel prediction marker and sensitizing target for radiotherapy. *Int J Radiat Oncol Biol Phys* 2010; **78**: 246 - 256.

107 Skipworth RJ, Deans DA, Tan BH, Sangster K, Paterson-Brown S, Brown DA *et al*. Plasma MIC-1 correlates with systemic inflammation but is not an independent determinant of nutritional status or survival in oesophago-gastric cancer. *Br J Cancer* 2010; **102**: 665 - 672.

108 Staff AC, Bock AJ, Becker C, Kempf T, Wollert KC, Davidson B. Growth differentiation factor-15 as a prognostic biomarker in ovarian cancer. *Gynecol Oncol* 2010; **118**: 237 - 243.

109 Koopmann J, Buckhaults P, Brown DA, Zahurak ML, Sato N, Fukushima N *et al*. Serum macrophage inhibitory cytokine 1 as a marker of pancreatic and other periampullary cancers. *Clin Cancer Res* 2004; **10**: 2386 - 2392.

110 Koopmann J, Rosenzweig CN, Zhang Z, Canto MI, Brown DA, Hunter M *et al*. Serum markers in patients with resectable pancreatic adenocarcinoma: macrophage inhibitory cytokine 1 versus CA19-9. *Clin Cancer Res* 2006; **12**: 442 - 446.

111 Selander KS, Brown DA, Sequeiros GB, Hunter M, Desmond R, Parpala T *et al*. Serum macrophage inhibitory cytokine-1 concentrations correlate with the presence of prostate cancer bone metastases. *Cancer Epidemiol Biomarkers Prev* 2007; **16**: 532 - 537.