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Inhibition of human androgen-independent PC-3 and DU-145 prostate cancers by antagonists of bombesin and growth hormone releasing hormone is linked to PKC, MAPK and *c-jun* intracellular signalling

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Abstract

Bombesin/gastrin-releasing peptide (BN/GRP) antagonists RC-3940-II and RC-3940-Et, and growth hormone-releasing hormone (GHRH) antagonists MZ-J-7-118 and RC-J-29-18 inhibit the growth of human androgen-independent PC-3 and DU-145 prostate cancers in nude mice. Additive inhibitory effects were observed after treatment with both classes of analogs. In the present study, we investigated the effects of these antagonists on intracellular signalling pathways of protein kinase C (PKC), mitogen activated protein kinases (MAPK) and *c-fos* and *c-jun* oncogenes that are involved in tumour cell proliferation. In PC-3 tumours, antagonists of BN/GRP and GHRH decreased significantly the expression of PKC isoforms alpha (α), eta (η) and zeta (ζ) and increased that of delta (δ) PKC protein. MAPK was not detectable. In DU-145 tumours, which constitutively express MAPK, all treatments strongly decreased the levels of p42/44 MAPK. Treatment with the antagonists tended to reduce m-RNA for *c-jun* in both tumour models. In proliferation assays *in vitro*, inhibitors of PKC and MAPK diminished growth of DU-145 and PC-3 cells. These findings suggest that antagonists of BN/GRP and GHRH inhibit the growth of androgen-independent prostate cancer by affecting intracellular signalling mechanisms of PKC, MAPK and *c-jun*.

Keywords: Prostate cancer; PKC isoforms; MAP-kinases; *c-fos*, *c-jun*; PC-3; DU-145

1. Introduction

Prostate cancer is a significant health problem among men in the Western world [1,2]. Whereas many patients with organ confined disease can be cured by radical prostatectomy or radiation therapy, a significant number will develop local recurrence and disseminated, metastatic disease. Androgen deprivation is an established treatment for advanced prostate cancer, but a relapse due to the development of androgen independence is frequently observed. The failure of androgen deprivation therapy could be due to amplification, loss or changes in the specificity of the androgen receptor. It has been shown that growth of advanced prostate cancer can be activated by a wide spectrum of other steroid hormones, non-steroidal antiandrogens and various growth factors [3].

Several growth factors are involved in growth of androgen-independent prostate cancer signal through

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protein kinase C (PKC) pathway [4,5]. The PKC family consists of several isoforms: the conventional (α, βI, βII and γ), the novel PKCs (δ, ε, θ), and the atypical PKCs (ζ, λ and μ) [6]. PKC isoforms are serine/threonine kinases involved in a wide range of physiological and pathological processes including differentiation, proliferation, apoptosis, neoplastic transformation and gene expression. The expression of PKC isoforms has been investigated in different prostate cancer models [7–9] and activation of PKC and its downstream targets is considered to be involved in the development of androgen-independent prostate cancer. Recently, specific functions of each isoform of PKC have been described and the evaluation of isoforms for use as targets of drug action was initiated [10]. ISIS 3521, an antisense oligonucleotide inhibitor of PKC-α, is in clinical trials for the treatment of hormone refractory prostate cancer [11]. The phospholipid signalling cascade includes activation of phospholipase C-β, PKC and finally mitogen activated protein kinase (MAPK), which after phosphorylation is translocated into the nucleus and increases the expression of immediate-early oncogenes such as *c-fos* and *c-jun* [10,12]. MAPK is important in determining the cellular response to several types of stimulation [13] and activated MAPK is frequently found in advanced prostate cancer [14]. The androgen receptor is regulated by phosphorylation and cross-talk with several signalling pathways, including PKC, PKA and MAPK [15]. Recently, it has been demonstrated that MAPK is constitutively activated in DU-145 cells, but not in other cell lines and that the high level of MAPK activity in the DU-145 cell line is linked to the effect of paracrine/autocrine growth factors [16,17].

Peptide growth factors including bombesin/gastrin releasing hormone (BN/GRP), growth hormone-releasing hormone (GHRH) and their receptors have been found in surgical specimens from patients with locally advanced prostate cancer [2,18,19] and in experimental human prostate cancer lines [2,18,20–24]. However, splice variants of GHRH receptors expressed by prostatic and other cancers are different from the pituitary isoform [2].

In previous studies, we showed that antagonists of BN/GRP and GHRH effectively inhibit the growth of various human experimental cancers including prostate cancer and reduce the concentration and receptor levels of tumoural growth factors [2,22,24,25]. The BN/GRP receptors are members of the G-protein coupled receptor super family and the signal transduction pathways involve the activation of phospholipase C, generation of inositol triphosphate, the release of intracellular calcium, and the activation of PKC [26]. Bombesin mediated mitogenesis can be blocked by different BN/GRP antagonists through interrupting the signal transduction process at various post-receptor levels [27]. This mitogenic block is mediated by uncoupling the receptor from its signalling system and is associated with down-regulation of PKC [27]. The PKC pathway could also be an important signalling system involved in the action of GHRH on its pituitary receptors as shown in studies on the control of GHRH secretion from ovine somatotropes [28]. It has also been observed that activation of pituitary receptors for GHRH produces a phosphorylation of MAPK and increases the levels of *c-fos* protein [29].

Nevertheless, the intracellular signalling pathways in prostate cancer involving PKC/MAPK affected by GHRH antagonists have not been studied previously. In H-69 human small-cell lung carcinoma, tumour inhibition by antagonists of BN/GRP and of GHRH is correlated with an inhibition of the PKC-MAPK-*c-fos*/*c-jun* signalling pathway [6]. Recently, we showed that BN/GRP antagonists RC-3940-II and RC-3940-Et; GHRH antagonists MZ-J-7-118 and RC-J-29-18; as well as a combination of BN/GRP antagonist RC-3940-II with GHRH antagonist MZ-J-7-118, strongly inhibited the growth of PC-3 and DU-145 human androgen-independent prostate cancers xenografted into nude mice [25]. In order to elucidate the intracellular signalling mechanisms involved in the antitumour action of these antagonists of BN/GRP and GHRH, we have investigated in the present study whether they affect the protein levels of PKC isoforms and MAPK, as well as the expression of early oncogenes *c-fos* and *c-jun* in PC-3 and DU-145 tumours.

2. Materials and methods

2.1. Peptides and reagents

GHRH antagonists MZ-J-7-118 and RC-J-29-18 were synthesised in our laboratory by methods similar to those described [30]. The chemical structure of MZ-J-7-118 is [CH_3-$(CH_2)_6$-CO-Tyr^1, D-Arg^2, Phe(4-Cl)^6, Ala^8, His^9, Tyr(Et)^{10}, His^{11}, Abu^{15}, Nle^{27}, D-Arg^{28}, Har^{29}]hGHRH(1-29)NH_2, where Phe(4-Cl) is 4-chlorophenylalanine, Abu is α-aminobutyric acid, Nle is norleucine, Har is homoarginine. RC-J-29-18 is the analog of MZ-J-7-118 with a C-terminal ethylamide modification. The BN/GRP antagonist RC-3940-II, originally synthesised in our laboratory [31], was made and provided by Zentaris Gmbh (Frankfurt/Main, Germany) as D-24197. Its chemical structure is [Hca^6, Leu^{13}ψ(CH_2N)-Tac^{14}]BN(6–14), where Hca is desaminophenylalanine, and Tac is thiazolidine-4-carboxylic acid. BN/GRP antagonist RC-3940-Et, which is the analog of RC-3940-II with a C-terminal ethylamide modification, was synthesised in our laboratory as described for RC-3940-II [31]. For daily subcutaneous (s.c.) injection, the compounds were dissolved in 0.1% dimethylsulfoxide (DMSO) in 10% aqueous propylene glycol solution.