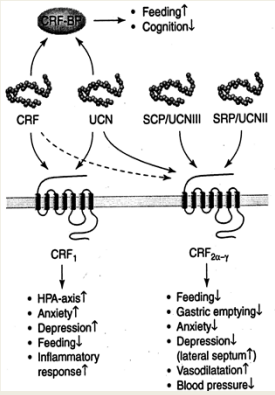
No.769

Activation of Corticotropin Releasing Factor receptor type 2 promotes hormone-dependent growth and migration of breast cancer cells

Koureta Maria[1], Papadaki-Anastasopoulou Artemis[1], Zarouchlioti Christina[1], Dekavallas Spyridon[1], Koffa Maria[2], Lambropoulou Maria[3], Chatzaki Ekaterini[1].

[1]Laboratory of Pharmacology, , [3]Laboratory of Histology/Embryology, Department of Medicine, [2]Laboratory of Cellular and Molecular Biology, Department of Molecular Biology and Genetics, Democritus University of Thrace, University Campus-Dragana, 68100 Alexandroupolis,Greece

Corresponding e-mail : m_koureta@yahoo.gr; achatzak@med.duth.gr



Introduction

Corticotropin Releasing Factor (CRF) system plays a central role in regulating stress responses. In parallel to their pituitary and CNS actions, CRF and its homologues Urocortins I, II and III, exert important direct biological effects in the periphery, via activation of two distinct GPCR receptors (Fig 1). CRF1 and CRF2 receptors present different pharmacological profiles to their ligands, and their expression is tissue-dependent. Many studies demonstrate that the CRF system could be involved in the growth and progression of human cancer (*Kaprara et al., 2010b*). Recently, we have shown the expression of both CRF receptor genes in human breast cancer biopsies and evaluated their prognostic/diagnostic potential (*Kaprara et al., 2010a*).

Fig 1: The CRF system: neuropeptide **CRF**, the homologues **urocortins I, II and III**, two types of membrane GPCR receptors **CRF1** and **CRF2** and the binding protein **CRF-BP**.

Methods

We investigated the expression of CRF receptors in breast biopsies from patients diagnosed for primary breast adenocarcinoma by *immunohistochemistry* (Fig.2) and in the estrogen sensitive MCF-7 human breast cancer cell line by *immunofluorescence* (Fig.3). Expression of CRF receptors in MCF-7 was also examined by real-time *RT-PCR* (Fig 4) before and after estrogen (E2) treatment. We then examined the effect of Urocortin II, a specific CRF2 ligand, in combination to the known mitogen 17-β estradiol (E2) on MCF-7 growth and migration, using the *xCELLigence real-time cell monitoring system* (Fig 5).

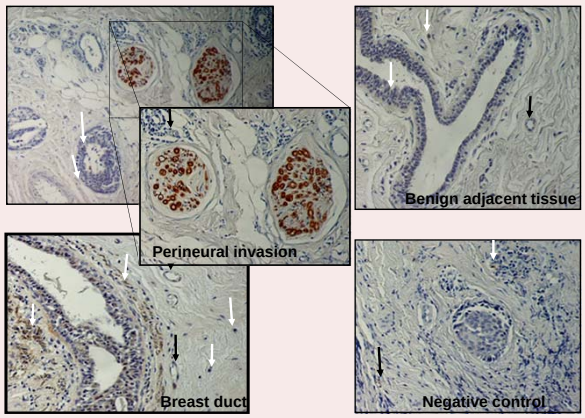
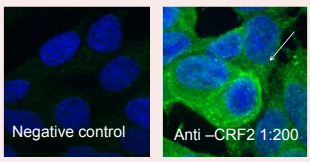
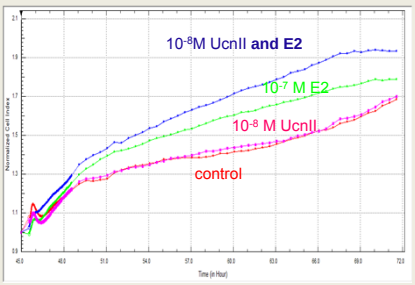


Fig 2: Immunohistochemical analysis of CRF2 receptor expression in human breast tumor biopsies. Intense positive receptor staining was localized in the cancer cells of **the perineural invasions,** in the blood vessels (black arrows) **and in periductal macrophages** (white arrows) but not of the cancerous implants and stroma.

Fig 3: Immunofluorescence for CRF receptor 2 in MCF-7cells.





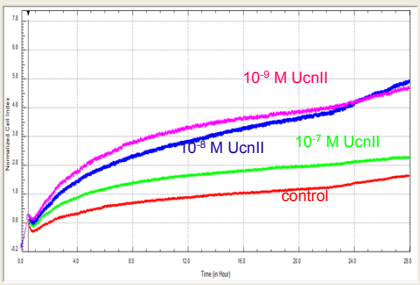
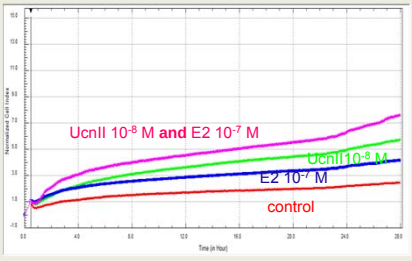
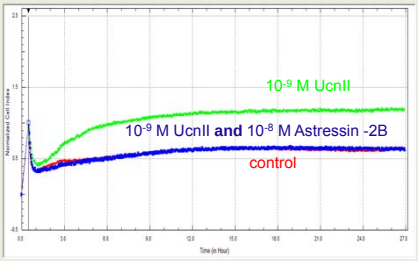


Fig. 5: a) Effect of UcnII on MCF-7 **cell Proliferation**: enhancement of E2 mitogenic activity

b) Effect of UcnII on MCF-7 **cell Migration:** dose-response





c) Effect of UcnII on MCF-7 **cell Migration**: enhancement of 17-β estradiol mitogenic activity

d) Effect of UcnII on MCF-7 **cell Migration**: Blocking by selective CRF Receptor 2 antagonist

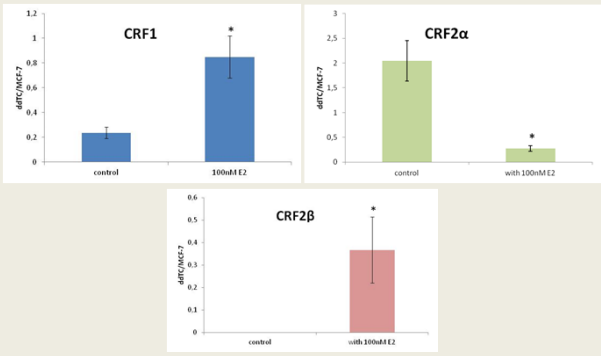


Fig 4: Expression of CRF receptors in MCF-7. Real-Time RT-PCR results for CRF1, CRF2α and CRF2β receptors in serum- free medium with or without E2.

Results

Histological mapping revealed positive CRF1 immunostaining in the cancerous implants and breast ducts, whereas CRF2 immunoreactivity was localized mainly in the perineural invasions. CRF1 and CRF2α receptor transcripts and proteins were found in MCF-7 cells. In serum-free conditions, E2 treatment increased significantly transcription of CRF1 and CRF2β, whereas CRF2α was decreased. Urocortin II did not alter MCF-7 cell growth at single administration, but it enhanced significantly the growth-promoting effect of E2, and this action was reversed by the selective CRF2 antagonist Astressin -2B. On the other hand, Urocortin II caused a dose- and time-dependent increase in the migration potential of MCF-7 cells, which again was inhibited by astressin2B. Urocortin II also enhanced the migration-promoting effect of E2.

Conclusions:

Both CRF receptors are found in breast cancer. CRF2 specific activation was shown to increase estrogen-dependent growth and migration of breast cancer cells. Further studies will be conducted to unfold molecular events involved in breast carcinogenesis via specific signaling pathways of the CRF system.

References

•Chatzaki E, Minas V, Zoumakis E, Makrigiannakis A., 2006; CRF receptor antagonists: utility in research and clinical practice (Review), Curr Med Chem.,13(23):2751-60.

•Dautzenberg FM, Hauger RL, (2002) The CRF peptide family and their receptors: yet more partners discovered (Review), Trends Pharmacol Sci. 2002, Feb;23(2):71-7.

•Graziani, G., Tentori, L., Muzi, A., Vergati, M., Tringali, G., Pozzoli, G., Navarra, P. (2007)Evidence that corticotropin-releasing hormone inhibits cell growth of human breast cancer cells via the activation of CRH-R1 receptor subtype. Mol. Cell Endocrinol. 264, 44–49.

•Kaprara A., Pazaitou-Panayiotou K., Chemonidou MC., Constantinidis TC., Lambropoulou M., Koffa M., Kiziridou A., Kakolyris S., Kortsaris A., Chatzaki E.(2010), Distinct distribution of corticotropin releasing factor receptors in human breast cancer. Neuropeptides. Oct;44(5):355-61. Epub 2010 Jul 13.

•Kaprara A., Pazaitou-Panayiotou K., Kortsaris A., Chatzaki E.(2010), The corticotropin releasing factor system in cancer: expression and pathophysiological implications. Cell. Mol. Life Sci. 67(8):1293-306. Epub 2010 Feb 9. Review.