Biologia, Bratislava, **60**/6: 711—715, 2005

Differences in endothelin-1 (ET-1) mRNA expression, ET-1 receptor down-regulation and signaling in "normal" human fibroblasts and cancer cell lines

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Abstract: When exposed to hypoxia human fibroblasts and human adenocarcinoma cells express high levels of mitogenic peptide endothelin-1 (ET-1). The ET-1 produced in cells binds almost exclusively to ET_A-subtype of endothelin receptors and acts as the key orchestrator of proliferation and malignant cell progression. ET-1 is cleared by proteases and specific endopeptidases. It is known that products of ET-1 degradation, the N-terminal endothelin fragments, were bound mostly to ET_B-subtype of endothelin receptors and showed most of the proliferative response produced by the parent peptide. The purpose of the current study was to reveal the role of human peptide ET-1 and N-terminally truncated peptide fragments in cellular signaling. The ET-1 expression, surface-membrane ET-1 receptor binding and resultant ET_A receptor down-regulation were studied in "normal" human vascular fibroblasts, in tumorigenic HeLa and HepG2 cells. An additional objective was to reveal the functional role of increased ET-1 mRNA expression in tumor cell lines and the role played by linear C-terminal ET-$1_{(6-21)}$ and ET-$1_{(8-21)}$ peptide fragments (BQ3020 and IRL 1620) in ET-receptor function. Our results showed a less intensive increase in ET_A receptor down-regulation (1.86 ± 0.23 fold, $P < 0.05$) in hypoxic tumorigenic cells and significantly reduced cellular signaling (proliferative index of hypoxic tumorigenic cells $= 0.89 \pm 0.04$, $P < 0.05$). The intensity of down-regulation of ET receptors after preincubation of endothelin fragments and ET-1 declined in sequence IIRL1620 > BQ3020 $\gg$ ET-1 and proliferation of HeLa cells was much more stimulated with ET-1 than with ET-1 fragments. This allowed us to propose a more precise mechanism of ET-1 production and binding in human cancer cells in contrast to normal human cells. The findings imply that endothelin ET_A antagonists may be considered in oncology for the treatment of endothelin-induced anomaly in cellular signaling in genitourinary tumors otherwise non-responding to conventional cytostatic therapy.

Key words: endothelin-1, endothelin receptors, ET-1 production, proliferation, HeLa cells, HepG2 cells, human cardiac fibroblasts, endothelin antagonists.

Abbreviations: DMEM, Dulbecco's modified Eagle's medium; ET-1, endothelin-1; FCS, fetal calf serum; HeLa, human adenocarcinoma cell line; HepG2, hepatocellular carcinoma cells; IR-ET-1, immunoreactive endothelin-1.

Introduction

Vascular fibroblasts are cells that participate in local inflammation, produce and are influenced by mediators that regulate differentiation and proliferation of cells. Tumorigenic cell lines contain malignant cells showing different degrees of differentiation, and hypoxia may significantly affect the spectrum of proteins expressed in cells. How malignant cells utilize their membrane receptor machinery to drive the neoplastic growth is still not clearly understood. Cells recognize autocrine/paracrine external signals captured by surface-membrane receptors and transform signal into a cellular response. Signals captured by two membrane-bound endothelin receptors, ET_A- and ET_B-, (Aramori & Nakanishi, 1992) are transduced inward to control cell proliferation and endothelin-1 (ET-1) gene expression. Abundant expression of ET-1, ET_A and ET_B receptors was identified in human tumors with epithelial morphology, in primary and metastatic ovarian carcinomas, small cell lung carcinomas and in breast cancer (Suzuki et al., 1989; Yamashita et al., 1999; Ahmed et al., 2000). However, the ET_A receptors in tumor cells may control more autocrine tumor growth (Bagnato et al., 1999) and ET_B receptors may protect tumor cells from hypoxia-associated apoptosis (Grimshaw et

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