Opioid-receptor gene expression and localization in cancer cells

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Received 05 July 2010; Accepted 24 August 2010

Abstract: This study examined the presence and cellular localization of three types of opioid receptors (MOR, DOR and KOR) in five human cancer cell lines: MCF-7, MDA-MB-231, HT-29, MGH-U1 and SH-SY5Y. Expression levels of opioid receptors were measured quantitatively using real-time PCR, and the localizations of the receptors in the cells were determined by immunocytochemistry. All three types of opioid receptors were present in each of the five cell lines examined. However, three of the cell lines (MCF-7, HT-29 and SH-SY5Y) showed significantly higher levels of MOR mRNA and protein than the other two types of receptors (DOR and KOR). Immunocytochemistry revealed that MOR, DOR and KOR receptors were predominantly present on the surface of cell membranes, although these receptors were also occasionally present in the cell cytoplasm.

1. Introduction

Opioid receptors belong to the superfamily of G protein-coupled receptors and couple to various effectors, such as adenylyl cyclase, calcium and potassium channels, via the pertussis toxin-sensitive G_i family of G proteins [1]. Pharmacological studies using highly selective ligands have classified opioid receptors into three main types, designated μ, δ and κ or MOR, DOR and KOR, respectively [2]. Molecular cloning studies over the past 15 years have confirmed this classification [3-6]. Each opioid receptor type has distinct binding properties for various opioid ligands and a distinct localization and distribution within the body [7,8].

Opioid receptors and their ligands are mainly found in the central nervous system, although they may also occur in peripheral organs and tissues [7,9]. Recently, the presence of opioid receptors has been reported in different cancer cells [reviewed in 10] and have been shown to be integral components of a wide variety of human and animal tumor cells, both in neural and non-neural tissues. The tumor types examined in previous studies were of ectodermal, mesodermal and endodermal origin, and some were malignant and others benign.

The occurrence of opioid receptors in tumor cells has prompted experimental studies investigating the effects of opioids on tumor growth in animals as well as the proliferation and survival of cancer cells in vitro. Unfortunately, the results obtained to date are controversial and often inconclusive. The most frequently used opioid in these studies was morphine, an exogenous ligand of MOR receptors. Reports on the effect of morphine in experimental and human cancers are conflicting; both growth-promoting and growth-inhibiting effects have been demonstrated [11-13]. In contrast to exogenous ligands, little is known about the role of endogenous opioid peptides in cancer cells.

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However, their influence should not be overlooked as they may have important effects during the earlier stages of a cancer. In order to investigate the influence of different opioids on cancer cells, we must first determine which opioid receptor types are present in these cells. In this study, we investigated the level of expression and the localization of mRNA and protein of MOR, DOR and KOR receptors in five human cancer cell lines. Our objective was to facilitate the choice of cell lines for further studies on the effects of opioid ligands in cancer cells.

2. Experimental Procedures

2.1 Cell cultures

The following human cancer cell lines were purchased from the European Collection of Cell Cultures (ECACC): two breast cancer cell lines, hormone-independent MCF-7 and hormone-dependent MDA-MB-231, colon cancer HT-29, neuroblastoma SH-SY5Y and bladder cancer MGH-U1. All cell lines were cultured according to the manufacturer’s instructions in culture mediums supplemented with gentamycin (5 µg/ml) and 10% heat-inactivated fetal bovine serum (both from Biological Industries, Haemek, Israel). Cells were maintained at 37°C in a 5% CO₂ atmosphere and grown until they were 80% confluent.

Cells from each cell line were seeded in 25 ml culture flasks in standard growth medium at a density of 2.5 x 10⁵ cells/flask. After 48 h, they were washed twice with PBS (Invitrogen, Carlsbad, CA, USA) with 0.01 M EDTA (Sigma-Aldrich, St. Louis, MO, USA), and later harvested by trypsinization. Cells used for mRNA isolation were frozen and kept at -80°C, whereas cells for immunocytochemistry were centrifuged and fixed with phosphate-buffered formaldehyde (Warszawskie Zakłady Farmaceutyczne Polfa, Warsaw, Poland) for 15 min at a pH of 7.4. After centrifugation, the excess formaldehyde was removed and cytological smears were prepared on SuperFrost Plus slides (Menzel-Glaser, Braunschweig, Germany). Cytological smears were fixed with 75% ethanol.

2.2 Quantitative RT-PCR assay

We used RT-PCR with an ABI Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) to quantify the expression of MOR, DOR and KOR mRNA and protein as well as the expression of glyceraldehyde 3-phosphate dehydrogenase (GAPDH), which was used as a house-keeping gene. Total cellular RNA (1 mg) was extracted from the following human cancer cell lines: MCF-7, MDA-MB-231, HT-29, SH-SY5Y and MGH-U1 using Trizol reagent (Invitrogen Life Technologies Inc., Rockville, MD, USA) and a single-step purification protocol [14]. RNA pellets were dissolved in water and their concentration and purity was determined by spectrophotometric readings at 260 and 280 nm. The relative amounts of the specific mRNAs were quantified by RT-PCR.

Extracted RNA was processed directly to cDNA using an Oligotex kit (Qiagen, Chatsworth, CA, USA). The cDNAs were amplified with forward and reverse primers, which are specific for human MOR, DOR, and KOR receptors and GAPDH. Primers were designed using PrimerExpress software (Applied Biosystems, Foster City, CA, USA). MOR primer sequences were 5’ GTCCTTATGCATTTCGATGAAC 3’ (forward) and 5’ CTG GAAGCAGAACTGCTGGTTG 3’ (reverse). DOR primer sequences were 5’ GGCTACGCAATAGCAGCCTGCAAC 3’ (forward) and 5’ CCGTCGCGATGG TCCCGGCGGTTGGCG 3’ (reverse). KOR primer sequences were 5’ GCTGCTCTCTGCCAGCTATTTCTC 3’ (forward) and 5’ GAGTCCGAAATACAGTTCAGGATCCTGC 3’ (reverse). GAPDH, the internal control, was amplified using primer sequences 5’-GTCGCTCTCTGCCAGCTATTTCT 3’ (forward) and 5’-GAGTCCGAAATACAGTTCAGGATCCTGC 3’ (reverse).

To create standard curves, we amplified 125, 100, 75, 50, 25, and 12.5 ng of the obtained cDNAs for GAPDH and for each of the target genes (MOR, DOR and KOR) in triplicate. In the same way, we amplified 100 ng of cDNA for GAPDH, as well as 100 ng of cDNA for each primer/probe combination. 50 µl of each sample containing the respective forward and reverse primers (0.3 µM) were prepared using qPCR™ Mastermix for SYBR Green I (Eurogentec S.A., Liege Science Park, Belgium) and amplified in a separate 96-well plate. All samples were incubated at 50°C for 2 min and at 95°C for 10 min, and then cycled at 95°C for 30 s, 56°C for 1 min, and 72°C for 1 min, for 40 cycles. SYBR Green I fluorescence emissions were captured and mRNA levels were quantified using the critical threshold (Cₜ) value. Controls without reverse transcription and with no cDNA template were included with each assay. Relative gene expression levels were obtained using the ∆∆Cₜ method [15]. For each determination, three independent experiments were performed in duplicate.

2.3 Immunocytochemistry

Cytological smears were put into PBS (Invitrogen, Carlsbad, CA, USA). Endogenous peroxidase activity was blocked by 0.3% hydrogen peroxide in distilled water for 30 min. The smears were rinsed with Tris-