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Opioids and migration, chemotaxis, invasion, and adhesion of human cancer cells ☆

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Abstract

This study was designed to examine the role of opioids on cell migration, chemotaxis, invasion, and adhesion, with an emphasis on whether the opioid growth factor (OGF, [Met^5]-enkephalin) or the opioid antagonist naltrexone (NTX) impacts any or all of these processes. Drug concentrations of OGF and NTX known to depress or stimulate, respectively, cell proliferation and growth were analyzed. Three different human cancers (pancreatic, colon, and squamous cell carcinoma of the head and neck), represented by seven different cancer cell lines (PANC-1, MIA PaCa-2, BxPC-3, CAL-27, SCC-1, HCT-116, and HT-29), were evaluated. In addition, the influence of a variety of other natural and synthetic opioids on cell motility, invasion, and adhesion was assessed. Positive and negative controls were included for comparison. OGF and NTX at concentrations of 10^{-4} to 10^{-6} M, and dynorphin A1-8, β-endorphin, endomorphin-1, endomorphin-2, leucine enkephalin, [D-Pen^{2,5}]-enkephalin (DPDPE), [D-Ala^2, MePhe^4, Glycol^5]-enkephalin (DAMGO), morphine, and U69,593 at concentrations of 10^{-6} M, did not alter cell migration, chemotaxis, or invasion of any cancer cell line. OGF and NTX at a concentration of 10^{-6} M, and incubation for 24 or 72 h, did not change adhesion of these cancer cells to collagen I, collagen IV, fibronectin, laminin, or vitronectin. Moreover, all other opioids tested at 10^{-6} M concentrations and for 24 h had no effect on adhesion. These results indicate that the inhibitory or stimulatory actions of OGF and NTX, respectively, on cell replication and growth are independent of cell migration, chemotaxis, invasion, and adhesive properties. Moreover, a variety of other exogenous and endogenous opioids, many specific for the μ, δ, or κ opioid receptors, also did not alter these biological processes, consonant with previous observations of a lack of effects of these compounds and their receptors on the biology of cancer cells.

Keywords: Neoplasia; Migration; Chemotaxis; Invasion; Adhesion; Pancreatic adenocarcinoma; Colon adenocarcinoma; Head and neck squamous cell carcinoma; In vitro

1. Introduction

The endogenous opioid peptide, [Met^5]-enkephalin, also termed the opioid growth factor (OGF), is a constitutively expressed native opioid that interacts with the OGF receptor (OGFr) to inhibit cell replication and growth during neoplasia, development, wound healing, and angiogenesis (Bisignani et al., 1999; Zagon and McLaughlin, 1993, 1999; Zagon et al., 2002, 2006; Blebea et al., 2000). OGF is an autocrine produced peptide that is secreted and has a direct and rapid action which is stereospecific, reversible, non-cytotoxic and non-apoptotic inducing, independent of serum, not associated with differentiative processes, and occurs at physiologically relevant concentrations (Zagon and McLaughlin, 2003, 2005; Zagon et al., 1996, 1999,

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2000a; McLaughlin et al., 1999a,b). With respect to neoplasia, OGF has anchorage-independent activity (Zagon and McLaughlin, 2004), and alters the progression of poorly- and well-differentiated cancers (McLaughlin and Zagon, 2006). OGF activity is not cell, tissue, or organ specific (e.g., Zagon et al., 1996, 1999, 2000b; McLaughlin et al., 1999a), and is targeted to the cyclin-dependent inhibitory kinase pathway in the G_0/G_1 phase of the cell cycle (Zagon et al., 2000c; Cheng et al., 2007). OGF also has been reported to influence tissue organization (Zagon et al., 1995). Addition of OGF either exogenously (Zagon et al., 1996, 1999; McLaughlin et al., 1999a) or by recombinant technology and delivery by a gene gun or transfection (Zagon et al., 2006, 2007; McLaughlin et al., 2007) excerbates the inhibitory pathways of OGF. Interruption of peptide-receptor interaction by sustained opioid receptor antagonism (e.g., the potent and long-acting opioid antagonist, naltrexone (NTX)), OGF-specific antibodies, or antisense/siRNA constructs for OGFr results in a substantial increase in cell number compared to control levels (Zagon et al., 2002, 2006; McLaughlin et al., 1999a,b), indicating the tonic and constitutive nature of OGF–OGFr interfacing.

The OGF–OGFr axis is known to repress a wide variety of neoplasias, whereas blockade of this axis leads to acceleration of oncogenesis (McLaughlin et al., 1999a,b; Zagon et al., 1996, 2000c, 1999). Moreover, the OGF–OGFr system is known to retard wound healing, while interruption of peptide-receptor interfacing stimulates wound repair (Klocek et al., 2007; Zagon et al., 2000d, 2006). One common denominator that would account for these observations is related to the modulation of cell migration, chemotaxis, and/or adhesion and, in the case of cancer, invasive properties. Thus, it could be hypothesized that one or more of these biological processes is instrumental in shaping the outcome of OGF–OGFr action and dysfunction. For example, retardation of neoplasias by upregulating the OGF–OGFr axis may be due in part to a decrease in cell motility (cell migration) or the ability to sense or react to environmental cues (chemotaxis, adhesive molecules). Acceleration in wound healing by disruption of OGF–OGFr interaction may be related to an increase in cell motility or a heightened response to the environment or biological substances.

The present study was designed to examine the hypothesis that the OGF–OGFr axis is dependent on the promotion or inhibition of cell migration, chemotaxis, adhesion, and/or invasion as a mechanism(s) for biological outcome. We have utilized standard methods to test the effects of opioids on these processes in three different human cancers documented to be regulated in proliferation by the OGF–OGFr axis: pancreatic, colorectal, and squamous cell carcinoma of the head and neck (McLaughlin et al., 1999a; Zagon et al., 1996, 1999). To examine whether other opioid peptides – synthetic and natural – can modulate cell migration, chemotaxis, invasion, and adhesion, we included a variety of opioids in our evaluation. These studies provide a comprehensive view of the role of the OGF–OGFr axis on the processes of cell motility and invasiveness in various cancer cells of human origin.

2. Materials and methods

2.1. Cell lines and cell maintenance

Human cancer cell lines utilized in this study included: PANC-1, Mia PaCa-2, and BxPC-3 pancreatic adenocarcinoma, UM-SCC-1 (SCC-1) and CAL-27 squamous cell carcinoma of the head and neck (SCCHN), and HCT-116 and HT-29 colon adenocarcinoma. All cell lines except the SCC-1 SCCHN cells were purchased from the American Type Culture Collection (Manassas, VA); SCC-1 cells were obtained from the Cancer Research Laboratory at the University of Michigan (Dr. Thomas E. Carey, Director). The characteristics of the cell lines have been described elsewhere (McLaughlin et al., 1999a; Zagon et al., 1996, 1999, 2000a,c; Zagon and McLaughlin, 2005). PANC-1, Mia PaCa-2, SCC-1, and CAL-27 cells were grown in Dulbecco's modified media, BxPC-3 cells were cultured in RPMI-1640 media with glutamate, and HCT-116 and HT-29 cells were grown in McCoy's 5 A media. For cell growth, the appropriate media was supplemented with 10% fetal calf serum (FCS), 1.2% sodium bicarbonate, and antibiotics (11 U/ml penicillin, 10 μg/ml streptomycin, 10 μg/ml neomycin). For experiments involving cell migration, chemotaxis, invasion, and cell adhesion, the appropriate media was supplemented with 1.2% sodium bicarbonate, and antibiotics (11 U/ml penicillin, 10 μg/ml streptomycin, 10 μg/ml neomycin) (= test media) plus either 0.1% bovine serum albumin (BSA) or 5% FCS. Cell cultures were grown in a humidified atmosphere of 5% CO_2/95% air at 37 °C.

Cells were maintained in T75 flasks until the day of the assay. For the migration and invasion assays the cells were harvested by trypsin, and for the adhesion assays the cells were scraped. Cell counting was performed using trypan blue exclusion staining. For all assays at least 3 wells/treatment were assessed. In general, 75,000–100,000 cells/well were utilized in the migration and chemotactic assays, 150,000–200,000 cells/well were analyzed in the invasion assay, and 50,000–70,000 cells/well were evaluated for adhesion properties.

2.2. Cell migration

Cell migration assays were performed using 8 μm pore size transwell inserts with membranes (BD Bioscience, Bedford, MA). In general, procedures followed