Morphine differently influences cell proliferation, migration and apoptosis in tumor cell lines endogenously expressing mu opioid receptor

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Opioid analgesics are widely used to treat moderate to severe cancer pain, both acute and chronic; furthermore, opioids are often administered together with anesthetics to reduce post-operative pain. An increasing body of evidence has accumulated demonstrating that mu opioid receptor (MOR) is overexpressed in several human malignancies; therefore, the role potentially played by opioid analgesics, as morphine, in the recurrence and metastatic rates of tumors, as well as any effect elicited by opioids on cell proliferation, apoptosis and cancer-related angiogenesis, have recently received considerable attention. Morphine may favor tumor progression indirectly by reducing mast-cells and natural killer (NK) activity, and directly by increasing cell proliferation and migration, promoting angiogenesis and antagonizing the cytotoxic effects elicited by anticancer agents as doxorubicin. On the other hand, morphine may also determine antitumor effects by increasing CD8+ T-cell activity and up-regulating Fas. Overall, the whole picture of opioid-mediated effects on cancer cells is still to be fully understood: data collected so far are controversial and seem to be influenced by many different variables as dose (high vs low), administration regimen (acute vs repeated), drug administered, cancer and cell type. Moreover, most of the research performed so far focused mainly on single tumor models or relied on retrospective studies carried out in humans; thus, highlighting the need of a comparative analysis carried out in parallel on different cancer cells investigated under similar experimental conditions.

Moving from these considerations, we aimed at characterizing any effect elicited by morphine on cell proliferation, migration and apoptosis of different tumor cell lines expressing MOR.

MOR mRNA and protein levels were preliminarily measured in each cell line via real-time PCR and saturation binding assays, respectively; HEK-293 cells not expressing MOR were adopted as negative control. [3H]thymidine incorporation, wound healing and Nexin® assay were employed to evaluate cell proliferation, migration and apoptosis, respectively: cells were exposed to 1 □M morphine (concentration reflecting the dose used in vivo) and assayed at different time points (0-48 h). HeLa cervix carcinoma displayed the highest MOR mRNA levels, followed by DAOY medulloblastoma, U87-MG astrocytoma, HT-29 colon carcinoma. Interestingly, MOR protein expression did not mirror the detected mRNA levels: DAOY cells displayed the highest MOR protein expression (1600 fmol/mg), followed by HeLa (702 fmol/mg) and U87-MG cells (604 fmol/mg); HT-29 cells showed the lowest MOR expression (180 fmol/mg). In these latter cells, 1 □M morphine tripled proliferation rate after 48 h, in a MOR-dependent way; interestingly, a shorter exposure to morphine (12 h) determined a transient, but significant increase in early apoptosis.

In HeLa cells, 1 □M morphine tripled proliferation rate after 24 h and increased migration by 2.6-fold with a peak at 18 h, being both effects MOR-dependent; a transient increase in early apoptosis was also observed after 12 h of exposure.

Morphine significantly increased U87-MG cell viability, migration and proliferation with peaks at 12, 18 and 48 h, respectively; these effects were MOR-mediated; furthermore, opioid antagonists significantly reduced U87-MG and HeLa basal migration, possibly unmasking an endogenous opioidergic tone contributing to cell migration.

Surprisingly, DAOY cells, albeit expressing the highest levels of MOR protein, did not show any altered cell proliferation, migration or apoptosis in response to morphine. Sequencing MOR in DAOY cells and studies in other medulloblastoma cell lines are currently ongoing and will be presented at the conference.

Taken together, these findings show that morphine significantly promotes cell proliferation and migration in different tumor models expressing MOR, being these effects time- and cell type-dependent.