EGF and neurotensin mediated proliferation in HT-29 colon cancer cells; defining a role for the scaffold protein NHERF-1

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Cancer of the lower intestine or colorectal cancer is the most common cancer in Australia, with approximately 1 in 20 Australians being diagnosed with some form of colorectal cancer each year. Current treatment relies on surgery combined with chemotherapy or radiotherapy. A deeper understanding of the molecular basis for cellular dysfunction in colorectal cancer is essential to identify new targets for therapeutic intervention. Epidermal growth factor (EGF) and neurotensin (NT) are important pro-proliferative factors implicated in colorectal cancer cell proliferation. Our research focuses on the roles of the Na^+/H^+ exchanger regulatory factor 1 (NHERF-1) as a PSD-95/Dlg/Zo-1 (PDZ) scaffold in mediating cell signalling in epithelial cells. This protein plays a major role in scaffolding macromolecular complexes involved in signal transduction. Importantly, NHERF-1 has been shown to interact with the EGF receptor to cluster the receptor at the cell surface (Lazar *et al.*, 2004). This study was undertaken to investigate the role of NHERF-1 in EGF and neurotensin (NT) mediated proliferation of colorectal cancer cells. The HT-29 cell line was derived from a primary human adenocarcinoma of the rectosigmoid colon and is widely used as a model for colorectal cancer. The basic strategy was to use lentiviruses to deliver siRNA against NHERF-1 and to study the effects of silencing NHERF-1 on EGF and NT evoked Ca^{2+} signalling and cellular proliferation using biochemical and spectrophotometric techniques. Cells were infected with the virus for 2 days, serum starved for 2 days and treated with EGF or NT for 24 hours. To investigate the binding of NHERF-1 to a putative PDZ binding domain in the C-terminal tail of the type 1 neurotensin receptor (NTR-1) co-immunoprecipitation was used. Treatment of the cells with either EGF or NT increased the rate of proliferation by 30±2% (EGF) and 17±5% (NT) (n=4). When the cells were exposed to EGF and NT together no synergism was observed, suggesting that the two mitogens may act *via* similar signalling endpoints. Infection of HT-29 cells with the lentivirus expressing siRNA against NHERF-1 reduced endogenous levels of the protein by 80±5% (n=3) as determined by Western blot. MTT-assays demonstrated that silencing of NHERF-1 also reduced cell proliferation of cells grown in serum containing medium by 57±12% (n=5). In addition, in cells silenced for NHERF-1, neither EGF nor NT had any proliferative effect. NTR-1 is a G-protein coupled receptor that signals *via* increases in intracellular Ca^{2+}. In cells silenced for NHERF-1, the peak increase in Ca^{2+} was reduced by 70±5% (n=5) of the control, as determined using the Ca^{2+} sensitive dye Fluo-3. However, co-immunoprecipitation of a GFP-tagged fusion protein expressing the C-terminus of NTR-1 showed no detectable interaction to NHERF-1 in HT-29 cells.

These data demonstrate a key role for NHERF-1 as a positive regulator of HT-29 cells in response to serum, EGF and NT. The exact molecular basis for this effect remains to be determined but suggests a role for macromolecular signalling complexes scaffolded by PDZ proteins. Inhibition of NHERF-1 may be a target for the design of more specific anticancer therapies.

Lazar, C.S., Cresson, C.M., Lauffenburger, D.A. & Gill, G.N. (2004) *Molecular Biology of the Cell*, **15**, 5470-5480.