Investigation into the regulation of p75^{NTR} by NGF and its pro-survival signaling in breast cancer cells

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Abstract

Triple negative breast cancers (TNBC) lack estrogen, progesterone and HER-2 receptors and thus are insensitive to current targeted therapies. However, TNBC are reported to secrete NGF and express its receptors, p75^{NTR} and TrkA, leading to NGF-activated autocrine signaling [1]. This provides a rationale for NGF as a potential therapeutic target for TNBC. We show here that NGF regulated the expression of p75^{NTR}. This is partially due to inhibition of processing of existing p75^{NTR} and partially due to an increase in p75^{NTR} mRNA transcription. This regulation of expression of p75^{NTR} by NGF requires Trk receptor. A differential sensitivity of the cells to apoptosis induction was observed with and without induction of p75^{NTR} by NGF.

1. Introduction

Nerve growth factor (NGF) is produced by over 80% of primary breast tumours tested, giving it a potentially broader target range than ER or HER-2. Pro-survival NGF signalling mediated by p75^{NTR} may contribute to the resistance of breast tumours to chemotherapy [2]. Normal breast cells do not secrete NGF, although they express both TrkA and p75^{NTR} receptors. Hence, NGF-targeted treatment would be more specific to breast tumour cells. Anti-NGF therapy has the possibility of increasing the effectiveness of chemotherapeutic drugs used as adjuvant therapies in breast cancer treatment.

2. Results and Discussion

A panel of breast cancer cell lines was screened for the expression of p75^{NTR} at mRNA and protein levels. MDA-MB-231 cells were found to express p75^{NTR} (data not shown) and were used throughout the experiments. Lysates from cells cultured over several days were probed with anti-p75^{NTR} and analyzed by western blot. This showed increase in expression of p75^{NTR} at later time points (data not shown). We hypothesized that this increase could be due to secretion of NGF into the medium. This was quantified by ELISA, which showed increased secretion with time in culture (data not shown). Similar results were obtained with exogenous NGF, as shown in Fig 2.1. To further confirm this NGF effect, NGF-neutralizing antibody was added in the presence of exogenous NGF. The increase in p75^{NTR} expression was diminished as shown in Fig 2.1. This effect was through NGF-dependent transcription/translation (Fig 2.1). MDA-MB-231 cells undergo constitutive processing of p75^{NTR} (data not shown), and this regulation of p75^{NTR} by NGF was due to partial inhibition of its processing (data not shown). Activated TrkA was also required for the NGF mediated regulation of p75^{NTR} (data not shown). A differential sensitivity of the cells to apoptosis induction was observed with and without induction of p75^{NTR} by NGF. This was determined by using Ro 08-2750 (an inhibitor of NGF binding to p75^{NTR}) in the presence or absence of apoptosis inducing agent at early and late time points where there was no to huge increase in secretion of NGF (from ELISA) corresponding to increase in expression of p75^{NTR} in the system (Fig 2.2).

2.1 Regulation of p75^{NTR} is through NGF-dependent transcription/translation

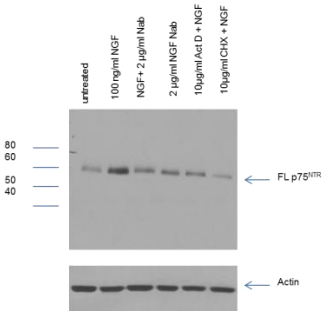

Fig 2.1: Regulation of p75^{NTR} expression is through NGF dependent transcription/ translation: Cells were treated with 100 ng/ml NGF in the presence or absence of 2 µg/ml NGF neutralizing antibody or 10 µg/ml Actinomycin D (Act D) or Cycloheximide (CHX) for 8 h. The cells were harvested for western blot -anti-p75^{NTR} (n=3).

2.2 NGF mediated increase in p75^{NTR} expression promotes protection of TNBC cells

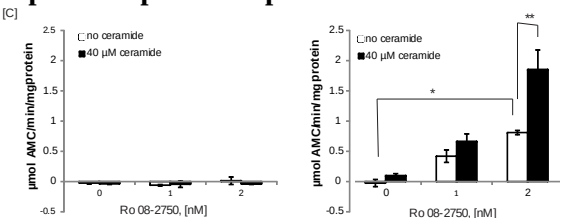

Fig 2.2: NGF mediated increase in p75^{NTR} expression promotes protection of TNBC cells: Cells were pre-treated with 1nM or 2 nM Ro 08-2750 for 1 h in the presence or absence of 40 µM C2-ceramide at different time points and apoptosis was determined using DEVDase assay (n=3); *p<0.05 on One-way ANNOVA followed by Tukey's post- hoc test.

3. Conclusion

This regulation of p75^{NTR} by NGF is linked to increased resistance of these cells to chemotherapeutic drugs. Hence, targeting NGF secretion and its interaction with p75^{NTR} in combination with conventional chemotherapeutic drugs will be of high therapeutic value. This work is funded by PRTLI 10.

4. References

[1] Dolle, L., et al,"Nerve growth factor overexpression and autocrine loop in breast cancer cells", *Oncogene* (2003) 22, 5592-5601.[2]Adriaenssens, E., et al. "Nerve growth factor is a potential therapeutic target in breast cancer", Cancer Research (2008) 68, 346-351