Role of HER4 in Neuroblastoma

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Abstract

Neuroblastoma (NBL) is the 4th most common pediatric tumor. Globally it constitutes 8-10% of all childhood cancers and is responsible of approximately 15% of all pediatric cancer deaths. It remains a challenge for pediatric oncologists, since long term survival for disseminated NBL is only less than 40%. Cancer cells' resistance to therapies might be one of the reasons for the failure in treatment; therefore there is an urge for finding novel treatment options. In this study we focused on HER4 and its role in neuroblastoma cell line CHP134. Results show that HER4 knockdown caused a significant decrease in cell proliferation. Also HER4 knockdown causes a decrease in tumorigenic potential. HER4 knockdown cell lines showed a slightly increase in sub G1 population when compared to nonsense control after drug treatment, suggesting that HER4 knockdown might be increasing chemo-sensitivity. We also found that different culture conditions affected HER4 expression. Monolayer culture showed an increase in HER4 expression as cell density increased, for sphere condition we obtained an up-regulation of HER4 when compared to monolayer culture. HER4 knockdown in sphere culture also promoted an increase in cleaved PARP, a marker of apoptosis, suggesting that HER4 knockdown in sphere condition increased apoptosis. From our results we propose that HER4 might be a therapeutic target for NBL treatment in the future. Further investigation is still required to understand how HER4 provides protection against stress.

Introduction

ERBB family of receptors tyrosine kinases, is comprised of EGFR, HER-2, HER-3 and HER4. It is known that EGFR and HER-2 activation occurs commonly in carcinomas, but less is known about HER-3 and HER4 in cancer . Richards *et al.* determined expression of the different ERBB family receptors in 20 NBL patient samples. Their findings showed that EGFR and HER4 were the predominant receptors expressed in NBL. This suggests that HER4 may play an important role in NBL and can be considered as a therapeutic target.

There are four isoforms of HER4 that vary within the juxtamembrane region (JM-a and JM-b) and within the cytoplasmic domain (CYT-1 and CYT-2).Only JM-a isoforms have been reported to have a proteolytic cleavage mechanism at the cell membrane that leads to nuclear translocation of an intracellular domain (4ICD). After binding of heregulin or following the activation of PKC by TPA HER4 ectodomain is cleavaged by TNFα-converting enzyme (TACE) and subsequent presenilin-dependent γ-secretase activity promotes membrane release of the 4ICD. To address the role of HER4 in neuroblastoma we evaluated HER4 expression in different densities as well as the effect of HER4 knockdown in neuroblastoma cell line CHP134.

Methods

1. ERBB4/Her4 expression was knocked down using short-hairpin RNA interference (shRNA).
2. Cells were cultured either in monolayer or sphere condition on Poly-HEMA pre-coated dishes.
3. Proliferation was measured by direct cell counting using Vi-cell cell counter.
4. Protein expression was measured by Western blot.
5. Tumorigenic potential was measured by soft agar clonogenic assay.
6. Cell cycle was measured by flow cytometry after staining cell with propidium iodide (PI).

Figure 1

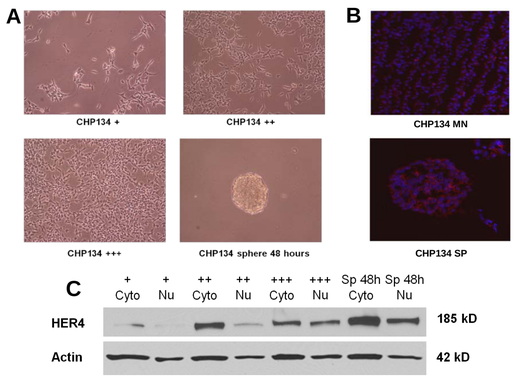

Figure 1. (A) CHP134 cells were seeded in sphere culture for 48 hours and at different densities. (B) Immunofluoresence of HER4 expression was obtained. Red dye indicates HER4 expression, this suggests an up-regulation of HER4 in sphere culture. (C) HER4 expression increases with density and is upregulated in sphere culture.

Figure 2

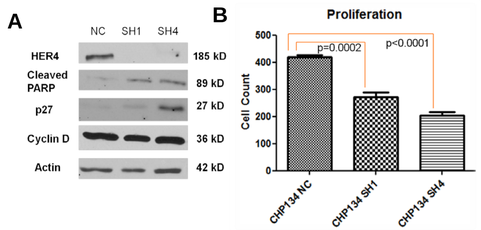

Figure 2. (A) Effectiveness of HER4 knockdown was verified by Western Blot. Results show that HER4 knockdown caused an increase in PARP cleavage, a marker of apoptosis. HER4 knockdown also promotes an increase of p27, a marker of cell cycle arrest. (B) HER4 knockdown caused a significant decrease in cell yield.

Figure 3

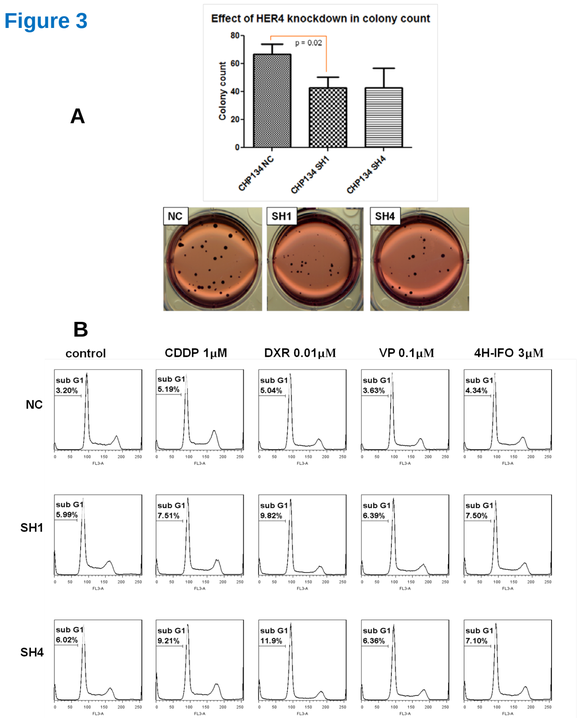

Figure 3. (A) Knockdown of HER4 causes a decreased colonogenic frequency in soft agar, suggesting decreased tumorigenic potential. (B) HER4 knockdown increased chemo-sensitivity in neuroblastoma cell line CHP134. [cisplatin (CDDP), doxorubicin (DXR), etoposide (VP-16), and 4-hydroxy-ifosfamide (4H-IFO)]

Figure 4

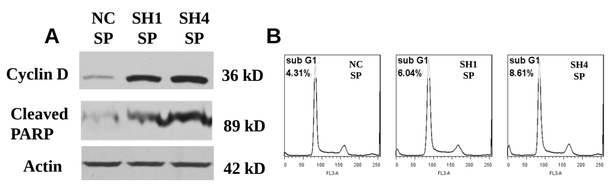

Figure 4. (A) In sphere culture CHP134-NC showed a cyclin D1 suppression. With HER4 knockdown there is higher cyclin D1 expression, and also an increase in cleaved PARP, a marker of apoptosis. (B) Flow cytometry shows an increase in sub G1 population for CHP134 HER4 knockdown cells in sphere condition. This suggests that HER4 knockdown increases apoptosis in sphere culture.

Results

- HER4 expression is density dependent showing upregulation at increased density and in sphere culture.
- HER-4 knockdown has several effects in CHP134:
  - Causes a significant decrease in proliferation
  - Increases apoptosis
  - Decreases tumorigenic potential
  - Increases chemosensitivity
  - Changes cell cycle regulation in monolayer and sphere culture.

Discussion and Conclusions

These results indicate that there is HER4 upregulation as density increases and in sphere culture. Knockdown of HER4 increased chemo-sensitivity and apoptosis in NBL cell line CHP134, and also decreased its tumorigenic potential. We suggest that HER4 might contribute to the malignant behavior in neuroblastoma and that it might play a protective role against cellular stress in neuroblastoma. From our results we propose that HER4 might be a therapeutic target for NBL treatment in the future.

References

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