ErbB family expression in osteosarcoma

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Abstract

Osteosarcoma (OS) is the most common bone malignancy among children and teenagers. Currently the gold standard treatment is surgery and chemotherapy; however the 5 year survival for these patients remains 60 to 70%. There has being tremendous interest in identifying markers of prognostic and therapeutic significance being a possible target the ErbB family of protein receptor tyrosine kinases (RYT). There are four members in the ErbB family, three (EGFR, Her2 and Her4) of which are expressed in primary OS cell lines. In this study we investigated the contributions of each of ErbB RYT to OS pathogenesis using cells genetically modified to knock-down the expression of either EGFR or Her-4. Cells were tweaked with ligands that target either EGFR (EGF) or Her-4 (heregulin, NRG1) to see which Ph ErbB-family member residues are activated, changed, or reduced. Expression of Ph EGFR was reduced in OS-O 3696 and Saos-2 3696 when compared to OS-O NC and Saos-2 NC. When stimulated with EGF, expression of Ph Her-2 decreased in OS-D 3696 when compared with parental D and OS-D NC; expression was reduced in Saos-2 3696 compared to Saos-2 NC. Expression of Ph Her-4 Ph was slightly increased in Saos-2 3696 compared with Saos-2 NC. We saw a slightly decrease in the expression of Ph EGFR and Ph Her-4 when stimulated with EGF in OS-D 1608 compared to OS-D NC. Moreover in OS-D 3847, the expression of both Ph EGFR and Ph Her-4 was significantly decreased while full length Her-4 expression was reduced in OS-D 3847 compared to OS-D 1608 and OS-D NC.

Introduction

Osteosarcoma (OS) is the most common bone malignancy among children and teenagers. It arises from mesenchymal bone forming tissue that produces malignant osteoid. The primary tumor is often found in the metaphyseal growth plates of long bones typically the distal femur and proximal tibia followed by the proximal humerus. It is important to note that these bones experience rapid cell division to facilitate the increase growth velocity during adolescence and that the stimulatory signals that allow for rapid growth could represent an inciting event in OS.

Currently the gold standard treatment for OS is surgery and chemotherapy; however the 5 year survival for these patients remains 60 to 70%. Up to 20% of patients presents, at the time of diagnosis, with metastatic disease. The survival rate of these patients drops to 10-30% and for patients with recurrent disease the survival rate is less than 20%. For this reason it is important to develop novel and effective approaches to treat OS patients.

Recent studies had focus in identifying tumor associated pathways and specific mediators of OS pathogenesis, progression, and prognosis. One possible prognostic and therapeutic target in OS is the ErbB family of protein receptor kinases. There are four members in the ErbB family: epidermal growth factor receptor (EGFR), Her-2, Her-3, and Her-4. They are composed of an extracellular binding region, a single membrane spanning region, and a cytoplasmic tyrosine kinase containing domain. Three groups of EGF-family peptides bind the ErbB receptors with selective specificity. Ligand binding initiates signaling that culminate in a variety of cellular responses including cell growth.

Studies done in the past were able to confirm expression of EGFR, Her-2, and Her-4 in primary and established OS cell lines. When using a pan-ERBB inhibitor, CI-1033, OS cells underwent growth inhibition and apoptosis suggesting a possible contribution of these 3 ErbB to OS pathogenesis. In this study we sought to investigate the contributions of the ErbB receptor tyrosine kinases to OS pathogenesis using cells genetically modified to knock-down the expression of either EGFR or Her-4.

Hypothesis

Reduced expression of any one given ErbB family member could affect or reduce expression and/or activation of other family members especially when stimulated with family member-specific ligands such as EGF.

Methods

Cell Culture

OS-D, OS-D NC, OS-D 3696, OS-D 1608, OS-D 3847, Saos2 NC and Saos2 3696 cells were cultured in high glucose (4.5 mg/ml) DMEM supplemented with 10% fetal bovine serum (FBS) and 1% AA. Cultures were maintained at 37˚ C in a 5% CO_2/95% air incubator.

Treatment

Primary osteosarcoma cells were grown in 100mm plates to ~80% confluence prior treatment. Cultures were treated with NRG-1 (25ng/mL) and Heregulin (25ng/mL) for 30 minutes and EGF (100ng/ml) for 15 minutes. Whole cell lysates were prepared by standard techniques.

Western Blot Analysis

Lysates were resolved by electrophoresis using an 8% SDS-PAGE and transferred to nitrocellulose membranes. The membranes were probed with EGFR PhY1068, Her-2 PhY1248, Her-4 PhY1162, or Her-4 Ab3275 overnight followed by incubation with appropriate secondary antibodies and detection with a SuperSignal Enhanced Chemiluminescence kit (Pierce, Rockford, IL). For sequential blotting, membranes were directly reprobed with proper antibodies (AKT PhS473, MAPK PhT202/204, and Stat5 PhY694)

Results

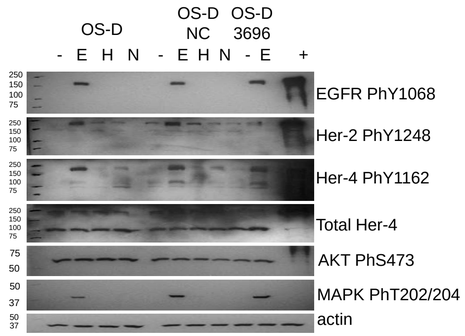

Figure 1. Expression of Ph EGFR, Ph Her-2, Ph Her-4, Total Her-4, Ph AKT, and Ph MAPK in OS-D, OS-D NC, and OS-D 3696

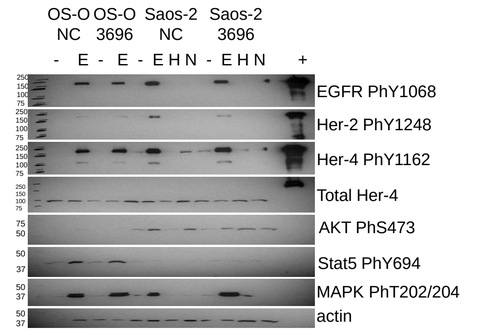

Figure 2. Expression of Ph EGFR, Ph Her-2, Ph Her-4 and Total Her-4, Ph AKT, Ph MAPK, and Ph STAT5 in OS-O NC, OS-O 3696, Saos-2 NC, and Saos-2 3696

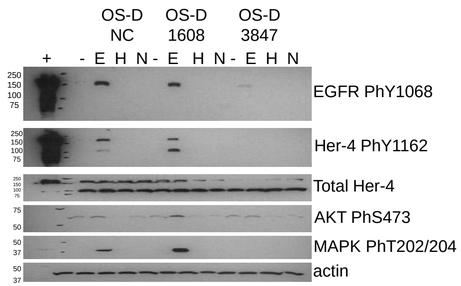

Figure 3. Expression of Ph EGFR, Ph Her-4, Total Her-4, Ph AKT and Ph MAPK, in OS-D NC, OS-D 1608, and OS-D 3847

Conclusions

- Expression of Ph EGFR did not change after stimulation with EGF in parental D, OS-D NC or OS-D 3696 while it was reduced in OS-O 3696 and Saos-2 3696 when compared to OS-O NC and Saos-2 NC.
- When stimulated with EGF, expression of Ph Her-2 did not change between parental D and OS-D NC but decreased in OS-D 3696; expression did not change in OS-O NC or OS-O 3696 but it was reduced in Saos-2 3696 compared to Saos-2 NC.
- Expression of Ph Her-4 Ph did not change upon stimulation with EGF in either OS-D or OS-O cell lines, and it was slightly increased in Saos-2 3696 compared with Saos-2 NC.
- As expected, expression of full length Her-4 was reduced in OS-D 3696 compared to OS-D NC and parental D and no expression was observed with OS-O NC, OS-D 3696, Saos-2 NC, or Saos-2 3696.
- We saw a slightly decrease in the expression of Ph EGFR and Ph Her-4 when stimulated with EGF in OS-D 1608 compared to OS-D NC.
- Moreover in OS-D 3847, the expression of both Ph EGFR and Ph Her-4 was significantly decreased while full length Her-4 expression was reduced in OS-D 3847 compared to OS-D 1608 and OS-D NC.
- The expression of Ph AKT did not change among groups while the expression of Ph MAPK increased in OS-D NC and OS-D 3696 compared to parental D.
- We did not see expression of Ph AKT in OS-O NC, or OS-O 3696. Expression of STAT-5 was slightly decreased in OS-O 3696 compared to OS-O NC while little expression was seeing in Saos-2 NC or Saos-2 3696.

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