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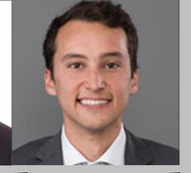

Expression of the ErbB Family of Receptor Tyrosine Kinases and Toll-like Receptor 4 on Oral Squamous Cell Carcinoma Cells: A Preliminary Study

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Background and Objectives

Epidermal growth factor receptor (EGFR) and its three related proteins are collectively known as an ErbB family of receptor tyrosine kinases (RTKs). It comprises of four distinct receptors: the EGFR (ErbB1/Her1), ErbB2 (neu/Her2), ErbB3 (Her3) and ErbB4 (Her4). It plays a critical function in the homeostasis of epithelial cells. It also drives cancer development.

Oral squamous cell carcinoma (OSCC) is considered the sixth most common cancer. It has been reported that an overexpression of EGFR correlated with progression and poor prognosis in SCC of the tongue. Therefore, targeted inhibition of EGFR is a promising approach to suppress signal transduction pathways which control tumor cell growth, proliferation, and resistance to apoptosis.

The role of bacteria in cancer, in particular initiation and progression, is well known. It has been reported that TLR4 activation by lipopolysaccharide (LPS) results in resistance to EGFR-targeted therapy in OSCC.

The overall goal of the study is to elucidate how oral bacteria influence the clinical outcome of the EGFR-targeted therapy in OSCC since the oral cavity harbors a diverse and complex microbial community. In this presentation, we reported an expression of ErbB family of RTKs and TLR 4 on OSCC cells.

Materials and Methods

OSCC cells

HSC-2, HSC-3, and HSC-4 human OSCC cells were purchased from JCRB Cell Bank (Ibaraki, Osaka Japan). They were maintained at 37°C, under 5% CO_2 in Dulbecco's modified Eagle's MEM medium supplemented with 10% fetal bovine serum (FBS), penicillin (100 units/ml), streptomycin (100 µg/ml) and L-glutamine (2 mM) (DME/10). For viability assay, HSC-3 cells were seeded in 48-well plates at a density of 1.0 x 10^5 cells per well in 1 ml of DME/10 medium 24 hours before the LPS challenge.

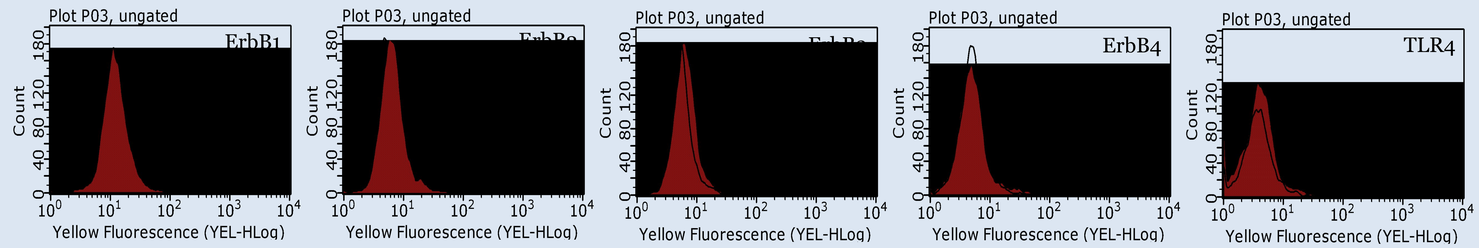
Analysis of Basal Expression Level of ErbB family proteins and TLR4

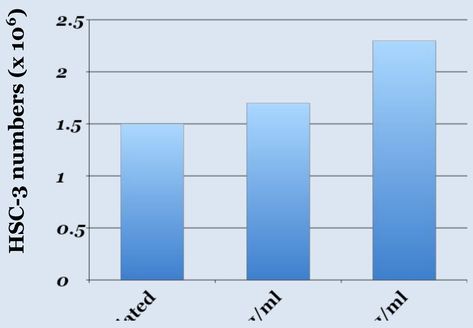
HSC-2, HSC-3, and HSC-4 cells were stained with ErbB1, ErbB2, ErbB3, ErbB4, or TLR4 for 60 min at 4°C. All analyses were performed on a guava easyCyte 8HT, using the InCyte 2.7.

Cell Viability Assay

The trypan blue exclusion test was used to determine the number of viable cells using Countess™.

Results





Numbers of HSC-3 cells in response to LPS stimulation for 24 hours

Conclusion and Future Directions

All OSCC cells tested expressed ErbB1, ErbB2, and ErbB3. HSC-3 and HSC-4 also expressed TLR4. As future directions, we will first examine whether or not LPS stimulation affects ErbB family protein expression (i.e., upregulation or downregulation). We hypothesized that the receptors will be upregulated since HSC-3 cell proliferated in response to LPS. Unveiling the crosstalk between EGFR and TLR4 in EGFR-targeted therapy in OSCC may potentially provide us with better therapeutic approach to target EGFR.