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MedChem & CADD 2016: VERO stable cell lines expressing full-length human epidermal growth factor receptors 2 and 3: Platforms for subtractive phage display

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Cross-talk between human epidermal growth factor receptor 2 and 3 (HER2 and HER3) may potentially contribute to therapeutic resistance in human breast cancer. Subtractive phage display allows highly specific selection for antibody fragments directed against cells surface HER2 and HER3. The strategies to select conformation- and activation-specific antibodies against HER2 and HER3 require tightly regulated HER2 and HER3 expressing cells that allow controlled activation/ inactivation of these receptors during panning procedures. To achieve this, first, we found that VERO cell line is an appropriate cell line for heterogeneous expression of HER2 and HER3, and then we established a panel of VERO stable cell lines expressing high levels of HER2 and HER3 alone and in combination. The cell line established here; not only provided platforms for phage display-based methods but also could be used in any HER-related studies and drug discovery.

The epidermal growth factor is a transmembrane protein and it belongs to the ErbB receptor family, a subfamily of four closely related tyrosine kinase receptors: EGFR (ErbB-1), HER2 / neu (ErbB-2), Her 3 (ErbB-3) and Her 4 (ErbB-4). In many types of cancer, mutations that affect the expression or activity of EGFR could cause cancer. Deficient signaling of EGFR and other receptor tyrosine kinases in humans is associated with diseases such as Alzheimer's disease, while overexpression is associated with the development of a wide variety of tumors. Interruption of EGFR signaling, either by blocking EGFR binding sites on the extracellular domain of the receptor or by inhibiting the activity of intracellular tyrosine kinase, can prevent the growth of tumors expressing EGFR and improve the patient's condition.

ErbB2 has no known direct activation ligand, and may be in a constitutively activated state or become active during heterodimerization with other family members such as EGFR. EGFR undergoes a transition upon activation by its growth factor ligands. From an inactive monomeric form to an active homodimer, it undergoes a transition. Although there is evidence that preformed inactive dimers may also exist before ligand binding. In order to create an activated heterodimer, EGFR can pair with another member of the ErbB receptor family, like ErbB2 / Her2 / neu, in addition to forming homodimers after ligand binding.

The dimerization of EGFR stimulates its intrinsic activity of intracellular protein-tyrosine kinase. In the C-terminal domain of EGFR occurs, autophosphorylation of several tyrosine (Y) residues as a result. These include Y992, Y1045, Y1068, Y1148 and Y1173, as shown in the diagram opposite. This autophosphorylation induces activation and downstream signaling by several other proteins which associate with phosphorylated tyrosines via their own SH2 domains which bind to phosphotyrosine. These downstream signaling proteins initiate several signal transduction cascades, primarily the MAPK, Akt and JNK pathways, leading to DNA synthesis and cell proliferation.

These proteins modulate phenotypes such as cell migration, adhesion and proliferation. Activation of the receptor is important for the innate immune response in human skin. The kinase domain of EGFR can also cross phosphorylated tyrosine residues from other receptors with which it is aggregated, and can itself be activated in this way. EGFR is essential for the ductal development of mammary glands and EGFR agonists such as amphiregulin, TGF -α and heregulin induce ductal

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and lobuloalveolar development even in the absence of estrogen and progesterone.

The identification of EGFR as an oncogene has leads to the development of anti-cancer therapies for EGFR (called "EGFR inhibitors"), including gefitinib, erlotinib, afatinib, brigatinib and icotinib for lung cancer and cetuximab for colon cancer. More recently, AstraZeneca has developed Osimertinib, a third generation tyrosine kinase inhibitor. Many therapeutic approaches target EGFR. Cetuximab and panitumumab are examples of inhibitors of monoclonal antibodies. However, the former is of the IgG1 type, the latter of the IgG2 type; the consequences on antibody-dependent cell cytotoxicity can be very different. Zalutumumab, nimotuzumab and matuzumab are the other monoclonals in clinical development.

Amplifications of the EGFR gene are the most common amplifications found in glioblastomas. EGFR amplifications are relatively rare in oligodendrogliomas, while overexpression of the EGFR protein is a common characteristic of oligodendrogliomas. Reifenberger et al. studied 13 grade II oligodendrogliomas and 20 anaplastic grade III oligodendrogliomas for amplification of the EGFR gene. They observed an amplification of the EGFR gene in a single anaplastic tumor. However Reifenberger et al. also observed that overexpression of EGFR mRNA is relatively common in low and high grade oligodendrogliomas (six of 13 oligodendrogliomas and 10 of 18 anaplastic oligodendrogliomas).

Monoclonal antibodies block the binding domain of the extracellular ligand. The binding site being blocked, the signal molecules can no longer bind to it and activate tyrosine kinase.

Another method uses small molecules to inhibit the EGFR tyrosine kinase, which is on the cytoplasmic side of the receptor. Without kinase activity, EGFR is unable to activate, which is a prerequisite for binding of adapter proteins downstream. In appearance, by stopping the signaling cascade in cells that depend on this pathway for tumor growth, proliferation and migration are reduced. Examples of small molecule kinase inhibitors are gefitinib, erlotinib, brigatinib and lapatinib (a mixed inhibitor of EGFR and ERBB2).

There are several quantitative methods that use the detection of protein phosphorylation to identify inhibitors of the EGFR family. New drugs such as osimertinib, gefitinib, erlotinib and brigatinib directly target EGFR. Patients were divided into positive EGFR and negative EGFR, depending on whether a tissue test shows a mutation. EGFR-positive patients have shown a response rate of 60%, which exceeds the response rate for conventional chemotherapy, but many patients develop resistance. The T790M mutation and the MET oncogene are two main sources of resistance. The most common side effect of EGFR inhibitors, found in more than 90% of patients, is a papulopustular rash that spreads to the face and torso; the presence of the rash is correlated with the anti-tumor effect of the drug. In 10% to 15% of patients, the effects may be severe and require treatment.

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