

Epidermal growth factor receptor (EGFR) downmodulation assay

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INTRODUCTION

With this assay the disappearance of the receptor from the cell surface is followed upon long exposure with saturant EGF concentration. Alterations in the normal balance between degradation and recycling of the receptor can be unmasked. Data should be plotted as percentage of surface EGFR/initial amount at zero time point (Y-axes) versus time (X-axes). In HeLa cells, after 60 min almost all the EGFR is downmodulated, with less than 10% remaining at the cell-surface.

MATERIALS

Binding buffer

- DMEM
- 0.1% BSA
- 20 mM Hepes

Mild acid/salt wash buffer (pH 4.5)

- 0.2 M Na acetate pH 4.5
- 0.5 M NaCl

METHODS

1. Cells are plated into 24 well in order to have 90 percent of confluence the day after (in the case of HeLa, 100.000 cells). Cells are plated in triplicates for each time point, plus one well for the unspecific binding.
2. The day after, cells are serum-starved for at least 4 hours in binding buffer and are incubated for various times (120', 60', 30', 10', 0) with 100 ng/ml of EGF at 37 °C, rinsed with cold DMEM, and surface-bound EGF was removed by treatment with mild acid/salt wash buffer (pH 4.5) for 5 min. This mild acid/salt treatment removed more than 90% of the total ¹²⁵I-EGF bound to the cells at 4 °C without affecting cell permeability, or EGF receptor binding, internalization, and degradation.
3. The number of binding sites at the different time points is then determined by incubating the cells at 4 °C for 2h with 100 ng/ml of ¹²⁵I-EGF: 10 ng/ml of ¹²⁵I-EGF (Perkin Elmer) + 90 ng/ml of cold EGF (Peprotech).
4. Cells are washed 3 times with cold PBS and lysed in 1 N NaOH.
5. The lysate is collected and the radioactivity present is measured. This sample represents the amount of EGFR at the membrane at the different time points. Nonspecific binding is measured for each time point in presence of 10 ug/ml of unlabeled EGF, and generally is not more than 3-4% of the total counts and is subtracted from the total radioactivity.
6. After being corrected for non-specific binding, the rate of downmodulation is expressed as percentage of EGFR remaining at the cell surface respect to the initial amount (100%, calculated at time=0).

BIBLIOGRAPHY

Sigismund S., Argenzio E., Tosoni D., Cavallaro E., Polo S. and Di Fiore P.P. (2008) Clathrin-mediated internalisation is essential for sustained EGFR signalling but dispensable for degradation. *Dev.Cell.* 15(2): 209-19.