

# Resistance to EGFR-TKI induces cancer stem cell enrichment in tumorspheres of NSCLC cells

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**BACKGROUND:** The three-dimensional tumor spheroid culture system (tumorsphere) is a functional *in vitro* assay that more closely mimics the growth characteristics of Cancer stem cells (CSCs) *in vivo*. Specifically, tumorsphere culture is the method, predominantly used, that enable to enrich cancer cell lines in CSC. CSCs are a sub-population of cells within cancer tissues with tumor initiation, metastasis and staminal properties, and which are involved in resistance to chemotherapy (*Shackleton et al, 2006; Campbel et al, 2007; Tirino et al, 2013; Lee et al, 2016*).

**AIM:** In this work we set up a model of human NSCLC cells, A549, resistant to the tyrosine kinase inhibitor (TKI) for epidermal growth factor receptor (EGFR), gefitinib, and we performed the characterization of CSC properties in tumorspheres derived from resistant and wild type cells. Specifically, we performed:

1. Selection and characterization of two different clones of A549 NSCLC, resistant to gefitinib.
2. Set-up the tumorsphere cultures for wild type cells and resistant clones.
3. Investigation of CSC properties of tumorspheres in term of morphological features, and staminal markers.

## METHODS:

1. A549 NSCLC cells have been treated in monolayer for six months with 1 to 10  $\mu$ M gefitinib (EGFR-TKI). Acquired resistance to the drug was evaluated by MTT assay.
2. In resistant and wild type cells, tumorspheres were performed as reported (*Han et al. 2013*);).
3. Morphological data (dimensions and number) of tumorspheres have been acquired by an inverted microscope and quantified with ImageJ. Staminal markers such as CD133, c-Myc, SOX2, ALDH1, have been analyzed by Western Blot.

## RESULTS:

1. Resistant A549 clones formed more (in number) and bigger (in surface area) tumorspheres compared to the tumorspheres formed by wild type cells.
2. Similarly, staminal markers were upregulated in tumorsphers from resistant clones compared to wild type cells.

**CONCLUSIONS:** This study indicates that resistance to the EGFR-TKI induces an enrichment in CSC in tumorsphere culture of A549.

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