Fibroblast growth factor receptors (FGFRs) induce intracellular signaling networks that tightly regulate cell proliferation, survival, migration, and differentiation. FGFR type 1 (FGFR1) gene amplification has been identified as one of the key potentially actionable targets in squamous cell lung carcinoma (SqCLC), given FGFR1 role in oncogenesis.

Equivocal results from the pre-clinical and clinical studies point at the necessity of efficient and reliable predictive identification of potential candidates for therapy with FGFR1 inhibitors [1].

FGFR1 amplification and protein overexpression has been reported respectively in 10-22% [2,3] and 10-41% [2,4,5] patients with squamous cell lung cancer (SqCLC).

Published data regarding correlation between FGFR1 protein expression and FGFR1 gene amplification are discordant [2]. Our previous analyses demonstrated relatively low intra-tumor heterogeneity of both markers in analyzed SqCLC cases [3].

Here we present results from retrospectively and prospectively analyzed SqCLC tumor samples in regards to FGFR1 protein expression and FGFR1 gene amplification, as well as concordance of both markers.

**RESULTS**

In 18/111 (16.22%) SqCLC tumors FGFR1 amplification was detected

- the average FGFR1 gene copy number per cell ranged from 1.23 to 13.97 (mean ± SD: 3.48 ± 1.88)
- the mean FGFR1/CEN8 ratio was 1.28 ± 0.59 (range: 0.53–4.35).
- the mean content of tumor cells with ≥5 FGFR1 copies was 7.51% ± 10.73% (range: 1.67 – 36.67).
- the mean content of tumor cells with large clusters of FGFR1 was 11.04% ± 17.30% (range: 1.67 – 90.00).

31 samples presented FGFR1 protein expression (staining intensity ≥1), but only 18/111 (16.22 %) was IHC positive (staining intensity ≥2).

**CONCLUSIONS**

There was no correlation between FGFR1 amplification and FGFR1 protein overexpression (P = 0.0003; r = 0.3369) in whole group of SqCLC tumors (n=111).

**MATERIALS & METHODS**

- Formalin-Fixed Paraffin-Embedded (FFPE) surgical samples from SqCLC patients (n=111).
- The study was approved by local ethical committee.

**METHODS**

The analysis of FGFR1 gene copy number was performed by fluorescence in situ hybridization (FISH) method using probes specific for the 8p12 locus and the chromosome 8 centromere (CEN8) (ZytoLight® SPEC FGFR1/CEN 8 Dual Color Probe and ZytoLight® FISH Tissue-Immunoprint Kit, ZytoVision GmbH, Germany). A total sixty nuclei (20 contiguous tumor cell nuclei from 3 areas) were individually evaluated by counting green FGFR1 and orange CEN8 signals.

- FGFR1 amplification criteria [3]:
  - FGFR1/CEN8 signal ratio ≥ 2.0 or
  - the average number of FGFR1 signals per nucleus ≥ 6 or
  - ≥10% of tumor cells containing ≥5 FGFR1 signals or large clusters

The FGFR1 protein expression was determined by immunohistochemistry (IHC). Tissue slides were subjected to antigen retrieval in Target Retrieval Solution, pH 9 (DAKO/Agilent Technology; Denmark) with PT Link (DAKO/Agilent Technology; Denmark).

- Tissues were incubated with anti-FGFR1 rabbit monoclonal antibody (Cell Signaling Technology, clone D8E4, Danvers, MA, USA).
- Detection was done with EnVision™ Flex-System (DAKO/Agilent Technology, Denmark).
- Positive expression was defined as staining intensity ≥ 2 or ≥ 3 (graded from 0 to 3+) in ≥ 1% of the cancer cells.

**Statistical analysis**

Concordance between FISH and IHC results was assessed using the GraphPad Prism software version 5.03, using Spearman test.

Low consistency between FGFR1 gene amplification and protein expression in squamous cell lung cancer (SQCLC)

**Table 2.** Double positive (IHC+FISH+) (8/111; 7.21%) and inconsistent (IHC-+FISH+) (20/111; 18.02%) SQCLC samples identified

The Fish and IHC results were consistent in 81.98% SqCLC tumors (n=91):

- 83/111 (74.77%) tumors double-negative
- 8/111 (7.21%) tumors double-positive

20/111 (18.02%) results were discordant:

- 10/111 (9.01%) IHC- (IHC-) / FISH+(IHC-)
- 10/111 (9.01%) IHC+(IHC+) / FISH-(IHC-)

There was no correlation between FGFR1 amplification and FGFR1 protein overexpression (P = 0.0003; r = 0.3369) in whole group of SqCLC tumors (n=111).

**REFERENCES**