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

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BACKGROUND

- 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 carcinoma (SqCLC).
- Published data regarding correlation between FGFR1 protein expression and *FGRF1* 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.

MATERIALS & METHODS

PATIENTS' CHARACTERICTICS

Table 1. Clinicopathological characterictics of patients

Number of cases		111
Age in years (range)		66,83 (54-82)
Gender	female	40
	male	71
Stage	I	42
	II	42
	III	18
	IV	2
	no information	7
Grade	G1	1
	G2	78
	G3	28
	no information	4

MATERIALS

- 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) (Zyto*Light*® SPEC FGFR1/CEN 8 Dual Color Probe and Zyto*Light* ® FISH-Tissue Implementation 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 ≥15 *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.

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 ≥15 *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)

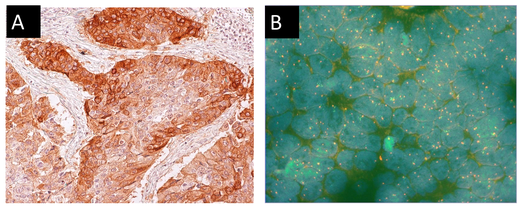

Figure 1. Representative examples of FGFR1 expression (A) and amplification (B) in SqCLC samples; green signal *FGFR1*, orange signal *CEN 8* .

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

Table 2. Double positive (IHC+FISH+) (8/111; 7,21%) and inconsistent (IHC+FISH-; IHC-FISH+) (20/111; 18,02%) SQCLC samples identified
samples fulfiil the criteria of FGFR1 amplification indicated in bold font

No	Slide no	Average *FGFR1*/CEN8 ratio	Average number of *FGFR1* signals per nucleus	Percentage of tumor cells containing		FISH results	IHC score	IHC results
				≥15 *FGFR1* signals	large clusters			
1	12	**2,77**	5,58	0,00	6,67	**FISH (+)**	3	**IHC (+)**
2	39	1,84	4,25	0,00	**13,33**	**FISH (+)**	2	**IHC (+)**
3	64	**2,66**	4,82	3,33	**21,67**	**FISH (+)**	3	**IHC (+)**
4	70	**2,65**	**13,97**	**36,67**	3,33	**FISH (+)**	3	**IHC (+)**
5	72	**2,74**	**6,05**	1,67	3,33	**FISH (+)**	2	**IHC (+)**
6	80	1,98	4,82	1,79	**10,00**	**FISH (+)**	2	**IHC (+)**
7	97	**2,83**	3,97	0,00	**25,00**	**FISH (+)**	3	**IHC (+)**
8	104	**2,35**	4,75	0,00	**90,00**	**FISH (+)**	2	**IHC (+)**
9	2	4,35	12,35	**31,67**	0,00	**FISH (+)**	1	IHC (-)
10	20	0,72	2,12	0,00	**10,00**	**FISH (+)**	0	IHC (-)
11	22	1,50	**6,35**	3,33	6,67	**FISH (+)**	1	IHC (-)
12	27	1,70	3,57	0,00	**40,00**	**FISH (+)**	0	IHC (-)
13	30	2,46	7,83	8,33	**35,00**	**FISH (+)**	1	IHC (-)
14	42	2,28	5,52	0,00	0,00	**FISH (+)**	0	IHC (-)
15	53	1,80	4,65	0,00	18,33	**FISH (+)**	0	IHC (-)
16	69	1,47	**6,20**	0,00	0,00	**FISH (+)**	0	IHC (-)
17	85	**2,00**	**7,93**	**10,00**	**33,33**	**FISH (+)**	0	IHC (-)
18	90	**2,44**	5,85	5,00	**33,33**	**FISH (+)**	1	IHC (-)
19	7	1,68	3,67	0,00	0,00	FISH (-)	3	**IHC (+)**
20	14	1,51	3,18	0,00	0,00	FISH (-)	2	**IHC (+)**
21	28	0,99	1,60	0,00	0,00	FISH (-)	3	**IHC (+)**
22	35	0,54	1,23	0,00	0,00	FISH (-)	2	**IHC (+)**
23	47	0,63	1,88	0,00	0,00	FISH (-)	3	**IHC (+)**
24	50	1,43	4,67	0,00	0,00	FISH (-)	2	**IHC (+)**
25	73	1,06	2,50	0,00	0,00	FISH (-)	2	**IHC (+)**
26	78	1,52	3,25	0,00	0,00	FISH (-)	2	**IHC (+)**
27	94	0,87	2,27	0,00	0,00	FISH (-)	2	**IHC (+)**
28	102	1,21	2,23	0,00	0,00	FISH (-)	3	**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).

CONCLUSIONS

The percentage of positive results (IHC and FISH) are consistent with presented by other authors.

FGFR1 amplification does not correlate with protein expression, because consistency of FISH and IHC results was observed mostly in double negative samples.

More detailed comparative evaluation of *FGFR1* gene expression or FGFR1 locus might allow more effective insight into the potential determinants of response to FGFR inhibitors.

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