Epidermal Growth Factor Receptor Copy Number Variations, but not EGFR or KRAS Mutations, Are Frequent in Lung Squamous Cell Carcinomas.

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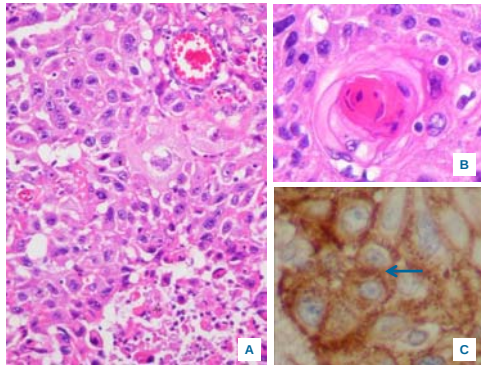

Figure 1: Key diagnostic features of SCCs. A: Solid pattern with squamous differentiation (H&E,x200), B: Squamous pearl formation (H&E,x200), C: EGFR Immunostaining emphasizing intercellular bridges (x400).

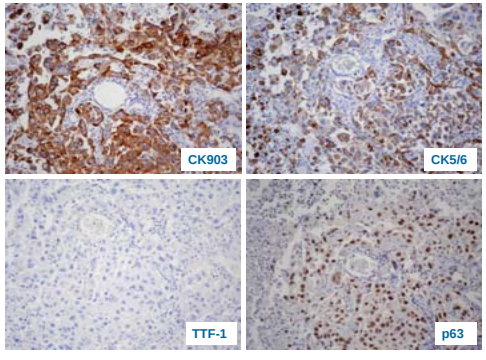

Figure 2: Characteristic immunoprofile of SCCs (x200).

BACKGROUND

Epidermal growth factor receptor (EGFR) mutations, and to a lesser extent EGFR copy number variations, have been correlated with response to EGFR tyrosine kinase inhibitors (TKIs). In contrast, KRAS mutations have been recently associated with resistance to TKIs. Most of these studies have been carried out on adenocarcinomas (ACs) due to the association of this histologic type with the presence of alterations in the EGFR gene pathway. The aim of this study was the molecular characterization of a series of lung squamous cell carcinomas (SCCs) and the possible implications on TKIs therapy.

DESIGN

A series of 47 surgically resected paraffin embedded SCCs were reviewed (Figure 1) and their histological classification further confirmed by IHC (CK7, CK5/6, CK903, CK20, p63 and TTF1). The characteristic SCC immunoprofile was P63 positive and TTF1 negative, this profile was reinforced by the expression of CK5/6 and CK903 (Figure 2).

The presence of mutations in exons 19 and 21 of EGFR and in KRAS exon 1 was analyzed by Sanger's sequencing and the incidence of EGFR copy number variations determined by dual-color FISH using a centromeric and a locus specific probe. Tumors were classified as FISH(+) when they presented high polysomy (≥4 gene copies in ≥40% of tumoral nuclei) or amplification (ratio gene:chromosome ≥2 or ≥15 gene copies in ≥10% of tumoral nuclei) (Figure 3). These results were then compared to those found in a series of 48 ACs and their statistical significance analyzed using Fisher's exact test. P-values were considered statistically significant when less than 0.05.

RESULTS

There were no EGFR or KRAS mutations found in this series of 47 SCCs, compared with the ACs series which presented EGFR mutations in 6 (12.5%) cases and KRAS mutations in 9 (18.7%) cases.

In contrast, there was a high percentage of SCCs cases (55%) presenting EGFR copy number variations, which is not statistically different from that found in the series of ACs (p=0.14). These FISH positive SCCs included 6 cases with EGFR amplification and 20 cases with high polysomy (Table 1).

CONCLUSIONS

- Our results confirm the absence of EGFR and KRAS mutations in lung SCCs observed in other series.
- The significant number of EGFR copy number variations observed and their possible correlation with TKIs sensitivity cannot be overlooked and should be further analyzed.
- This study suggests that FISH may be an appropriate methodology to assess the EGFR status of SCCs.

REFERENCES

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	SCCs (n=47)	ACs (n=48)	P value
EGFR FISH			
(+)	26	34	**0.14**
(-)	21	14	
EGFR mutations			
WT	47	42	0.03
Mutated	0	6	
KRAS mutations			
WT	43	31	0.0008
Mutated	0	9	
Not assessed	4	8	

Table 1: Univariate analysis to correlate molecular alterations with histologic type.

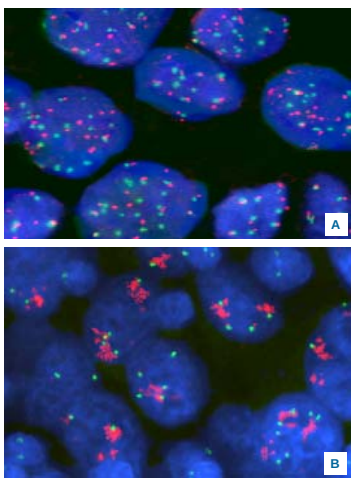

Figure 3: Examples of EGFR positive cases by FISH. A: High Polysomy, and B: Amplification. (x1000).

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