Figure 1. Scheme illustrating the effect of deregulation of the *EGFR* signaling pathway in a tumor cell .

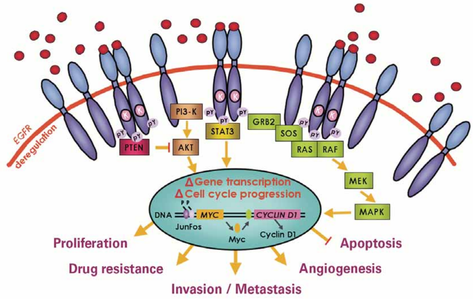


Figure 2. Criteria for the six FISH categories according to Cappuzzo et al. *J Natl Cancer Inst* 2005;97:643-55.

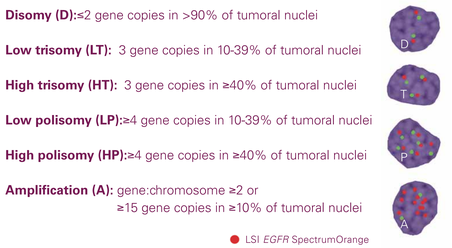


Figure 3. FISH-positive tumors exhibiting different patterns of gain in the *EGFR* gene copy number. (a) Amplification with a gene:chromosome ratio ≥2; (b) Amplification in tight gene clusters with ≥15 gene copies per tumoral nucleus.

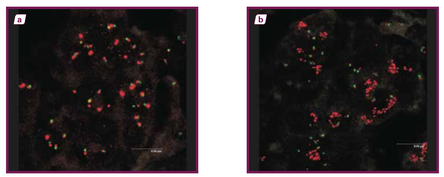
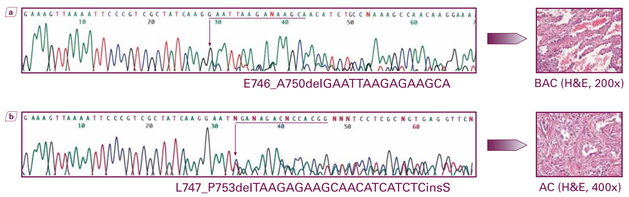


Figure 4. Exon 19 mutations of the *EGFR* gene found in the present study. (a) Bronchioloalveolar carcinoma with a 15 nt deletion; (b) Adenocarcinoma with a 21 nt deletion.



EPIDERMAL GROWTH FACTOR RECEPTOR IN NON SMALL CELL LUNG CANCER: GENE COPY NUMBER AND MUTATIONAL STUDY.

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INTRODUCTION

- Non small cell lung cancer (NSCLC) is the leading cause of cancer death worldwide for both men and women.
- Deregulation of the *EGFR* gene in NSCLC increases tumor cell growth and invasion **(Figure 1)**.
- Somatic activating mutations in the tyrosine kinase domain of the *EGFR* gene (exons 18-21) were found in tumors from patients with NSCLC that responded to therapy with tyrosine kinase inhibitors (TKIs), such as gefitinib and erlotinib.
- *EGFR* mutations occurred most frequently in women, in never smokers and in patients with adenocarcinomas rather than those with other histologic types, in agreement with the profile of TKI-responders.
- The frequency of *EGFR* mutations in Western populations seldom reaches 10%, whereas in Asian populations it overcomes 20%.
- Previous studies showed that patients with NSCLC who had *EGFR* mutations dramatically improved survival when treated with gefitinib.
- Recently, survival has been reported to significantly increase among patients treated with erlotinib that carried a high number of copies of the *EGFR* gene in comparison with those receiving placebo.

The present study was undertaken to examine the presence of *EGFR* mutations and its gene copy number in a series of NSCLC obtained from patients in the area of Barcelona, Catalonia, Spain.

PATIENTS AND TISSUES

Patients. A cohort of 83 patients with NSCLC from the area of Barcelona, Catalonia, Spain was used for this retrospective study. Demographic data on this cohort may be summarized as follows: 72 patients (87%) were males and 11 (13%) were females; median age was 63.4±10.1 years (range 42-83).

Tissues. Specimens were routinely fixed in 10% buffered formalin and embedded in paraffin. Representative hematoxylin and eosin-stained (H&E) sections of each case were examined microscopically by a pathologist, who selected viable representative areas of paired normal and tumor tissue for DNA extraction.

Concerning histopathology, tumors were classified as 32 adenocarcinomas (AC), 5 bronchioloalveolar carcinomas (BAC), 2 adenosquamous carcinomas (ASC), 36 squamous cell carcinomas (SCC), and 8 large cell undifferentiated carcinomas (LCUC).

METHODS

Tissue Macrodissection and DNA Isolation. Ten 5-µm-thick sections were used to perform manual scrapping. DNA was isolated using a proteinase K-phenol/chloroform protocol.

Quality Control. DNA quality was checked prior to specific studies by amplification of a 268 bp fragment of the human β–globin gene.

PCR and Sequencing Studies. PCR products were obtained from single reactions that spanned entire exons 18, 19, 20 and 21 of the *EGFR* gene. Mutational analyses were conducted by bidirectional fluorescence sequencing using the ABI PRISM® BigDye Terminator v1.1 Cycle Sequencing Kit.

Dual-color FISH Studies. *EGFR* gene copy number per cell was investigated using both a locus specific (LSI EGFR SpectrumOrange) and a centromeric probe (CEP7 SpectrumGreen) from Vysis. Tumors were classified according to the six categories defined by Cappuzzo et al (**Figure 2**). Samples with high degree of polysomy or amplification were considered FISH-positive.

Statistical Analyses. *EGFR* mutations and *EGFR* gene copy number were investigated for their association with clinicopathological features by Fisher's exact test. *p*-values were considered statistically significant when less than 0.05.

RESULTS

EGFR Gene Copy Number Status. By FISH, 76 out of 83 specimens (92%) were evaluable. Forty two cases (55%) were found to carry high EGFR copy number. Of those, 9 cases (21%) exhibited amplification (**Figure 3**) while 33 (79%) exhibited high polysomy. Out of the 34 cases with low gene copy number, 14 (41%) had disomy, 6 (18%) had low trisomy, 7 (21%) had high trisomy, and the other 7 (21%) had low polysomy.

Neither histologic type (AC including BAC *versus* other types) nor gender did significantly correlate with *EGFR* gene copy number.

EGFR Mutational Status. By sequencing, 71 out of 83 specimens (86%) were evaluable for exons 18, 19 and 21. Among these cases, 3 mutations (4%) were detected corresponding to large *EGFR* exon 19 deletions previously described (2 cases with delE746-A750 and one case with delL747-P753insS). Mutational analysis of paired normal tissue (lymph nodes) was done to confirm somatic origin for these mutations. A germinal 2754C>T nucleotide substitution previously reported in exon 21 was detected in one case that did not trigger aminoacid change (R836). Later on, mutational analysis of exon 20 was performed and 67 cases resulted evaluable, none of which exhibited mutation.

Somatic mutations were found in two AC and one BAC, all cases from patients who carried high *EGFR* gene copy number (2 with high polysomy and one with amplification) (**Figure 4**). The 3 mutations were detected in tumors from women, which represents that 27% of female patients in our series exhibited *EGFR* mutations.

CONCLUSIONS

- The rate of *EGFR* mutation occurred in our series of NSCLC is very low, in agreement with previous results obtained from other Western populations.
- The type of *EGFR* mutations found in our series as well as its clinicopathologic context (histological type and gender) is also in agreement with previous published results obtained from similar series.
- In our study, 55% of patients *versus* 4% had an altered *EGFR* tumor status when assessed by FISH and PCR-sequencing, respectively. Our results suggest that FISH is best choice to assist in discerning *EGFR* molecular profile of NSCLC, since it identifies more patients that are likely to benefit from TKIs therapies.
- Further studies should be conducted to elucidate the role of *EGFR* alterations in the prognosis of NSCLC so as to tailor individual treatments based on the *EGFR* molecular profile.

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