Highly sensitive real-time PCR for the detection of EGFR mutations in lung adenocarcinoma. Is it worth it?

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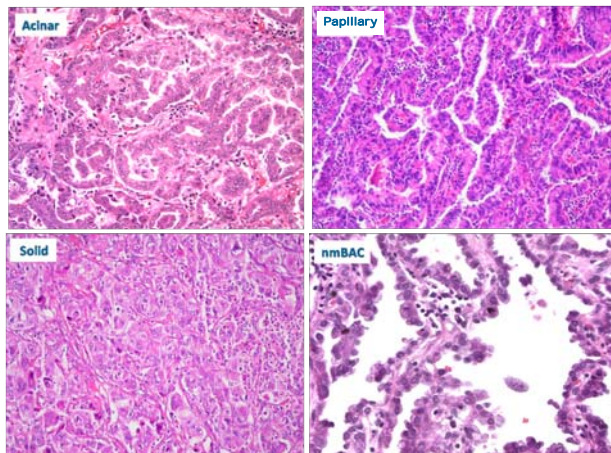
Figure 1. H&E sections of AC histological subtypes (Acinar, Papillary ,Solid (x200), and nmBAC (x400)).
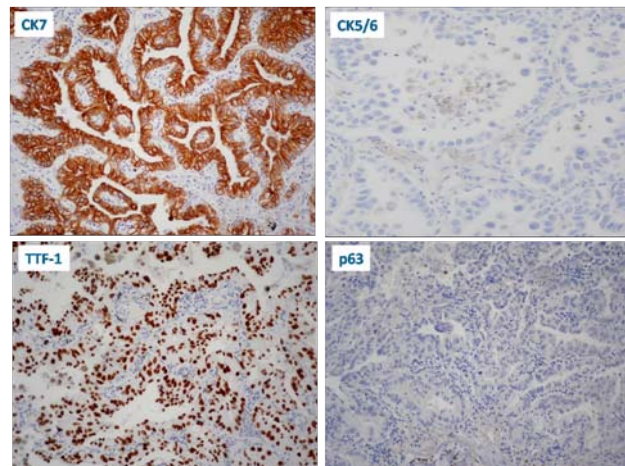

Figure 2. AC immunostaining for CK7, CK5/6, TT-1 and p63 (x200).

BACKGROUND

Mutations involving the epidermal growth factor receptor (EGFR) correlate with responsiveness to tyrosine kinase inhibitors (TKIs), which significantly improves patient survival in lung adenocarcinoma (AC). These mutations are described to be more frequent in non-mucinous lepidic adenocarcinomas (nmBAC). The unquestionable importance of determining the presence of these mutations, even in samples with a small tumor representation, has boosted the appearance of highly sensitive methods which would allow the detection of 1-5% mutated DNA. The aim of this study was to test the ability of a Real-time PCR kit to detect EGFR mutations in a series of lung AC considered wild-type by the Sanger's sequencing method.

METHODS

Patients. A cohort of 52 primary lung ACs were revised by two independent pathologists to establish histological type and immunoprofile. Demographic data on this cohort may be summarized as follows: 34 patients (65.4%) were males and 18 (34.6%) were females; median age was 64.6 years (range 42-82).

Tissues. Specimens were routinely fixed in 10% buffered formalin and embedded in paraffin (FFPE). Representative hematoxylin and eosin-stained (H&E) sections of each case were examined. Histopathological features were evaluated according to previously reported criteria (Figure 1). Ten 5μm thick sections of formalin-fixed, paraffin-embedded, tissues were used to perform manual scrapping. DNA was isolated using a proteinase K-phenol/chloroform protocol.

Immunohistochemistry: A panel of six monoclonal antibodies (CK7, CK5/6, 34βE12, CK20, p63 and TTF1) was used to determine AC immunoprofile (Figure 2).

PCR, Sequencing Studies and Real-time PCR. PCR products were obtained from single reaction for EGFR exon 19, 20 and 21. Mutational analysis was carried out by direct sequencing using the ABI PRISM® BigDye Terminator v1.1 Cycle Sequencing Kit and with the Real-time PCR kit *Therascreen EGFR PCR* (*Qiagen*).

DNA from FFPE cell lines: EGFR (ΔE746-A750/+) and EGFR (+/+) cell lines (*Horizon Diagnostics)* were used to asses the real sensitivity of Sanger's sequencing and Real-time PCR. Ratios of mutant *vs* WT DNA tested were : 20%, 10%, 5% and 1%.

Fluorescent in-situ Hybridization (FISH): was performed to assess the copy number status of EGFR gene using Vysis LSI EGFR SpectrumOrange/CEP7 SpectrumGreen Probe (*Abbot Molecular*).

RESULTS

Sanger's direct sequencing method allowed the determination of 7 cases with EGFR mutations (13.5%) and 45 EGFR wild-type tumor samples. *Therascreen* EGFR PCR kit detected 4 new cases with EGFR mutations, three of which showed 100% infiltrating nmBAC pattern and one acinar with 50% nmBAC component, increasing the global number of mutant cases to 11 (21.2 %). The most prevalent histological subtype in the mutated group corresponds to nmBAC pattern, being present in 8 of 11 (72.7%) cases. FISH analysis reveals 9 of 11 (81.8%) EGFR mutated cases being FISH positive, 2 by EGFR amplification and 7 with high polysomy (Table 1).

Sanger's sequencing allows the detection of 10% mutant EGFR (ΔE746-A750) from FFPE cell lines (Figure 3) while Real-time PCR kit *Therascreen* reaches as low as 1% mutant EGFR (Figure 4).

CONCLUSIONS

• The use of Real-time PCR in the global series increases the percentage of EGFR mutated samples detected by 7.7%.

• Considering that all 4 recovered cases have nmBAC component, the use of highly sensitive techniques in this subtype of AC should be recommended despite their substantial cost.

Histological Subtype	EGFR mutation		EGFR FISH Positive
	Sanger's Sequencing	*Therascreen*	
nmBAC (n=15)	4	4	12
Mucinous BAC (n=1)	-	-	1
Acinar (n=9)	-	-	7
Papilar (n=1)	-	-	-
Solid (n=13)	1	-	8
Mixed (n=8)	1	-	4
AC NOS (n=5)	1	-	4

Table 1. EGFR mutant and EGFR FISH status according to AC histological subtype.

20% √

10% ±

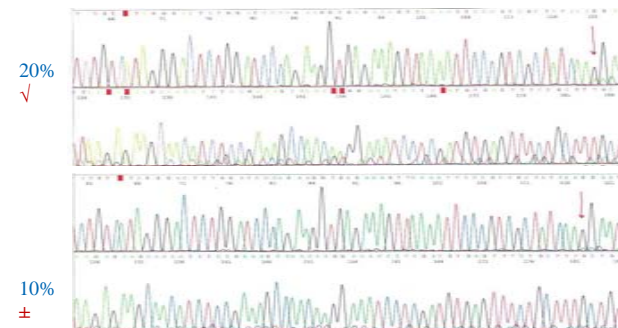**Figure 3.** Sanger's sequencing detection of 20% and 10% of mutant EGFR (ΔE746-A750) in a background of WT EGFR DNA from FFPE cell lines.
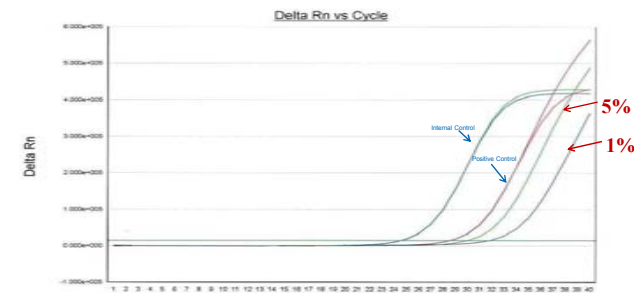

Delta Rn vs Cycle

5%
1%

Figure 4. *Therascreen* detection of 5% and 1% mutant EGFR (ΔE746-A750) in a background of WT EGFR DNA from FFPE cell lines.

References:
•Verdu et al. Abstract in Modern Pathology 2006; 19 (suppl. 3): 142.
•Travis WD et al. Journal of Thoracic Oncology, 2011; 6: 244-485..