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Gene mutations in small-cell lung cancer (SCLC): Results of a panel of 6 genes in a cohort of Italian patients

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ABSTRACT

Background: No target therapies are presently available in the treatment of small-cell lung cancer (SCLC). We investigated the presence of potentially drugable mutations in the *EGFR*, *c-MET*, *BRAF*, *KRAS*, *PDGFRa* and *c-KIT* genes in a retrospective series of SCLC from 2 Italian Institutions. Correlations with immunohistochemical, clinical and outcome features were evaluated.
Materials and methods: Genes were studied by direct sequencing of DNA extracted from formalin-fixed paraffin-embedded tissues. Immunohistochemical expression of TTF-1, p63, chromogranin, synaptophysin, CD56 and bcl-2 was assessed.
Results: Samples from 113 SCLC patients were analyzed. All cases were wild-type for *BRAF*, *KRAS*, *PDGFRa* and *c-KIT* (data available for 82 patients). Two (1.8%) patients were *EGFR*-mutated (exon 19 delE746-A750 and exon 21 L858R); both were females, non-smoker and had limited disease. Overall survival of *EGFR*-mutated patients was 21 months as compared to 11 months in wild-type. Five (4.4%) patients were *c-MET*-mutated (4 on exon 14: 2 R988C, 1 D990N, 1 D102Y; 1 on exon 17 R1166Q); all were smokers, 3 were males and 4 had extensive disease. Their OS was comparable to wild-type cases (12 vs. 11 months). *EGFR* and *c-MET* mutations were mutually exclusive. Gene mutations did not correlate with immunophenotype.
Conclusions: Targetable mutations are uncommon in SCLC. *EGFR*-mutated patients tended to be female and non-smoker and experienced a prolonged OS suggesting a possible positive prognostic effect. *c-MET* mutations did not affect survival. Target therapy might be considered in *EGFR* and *c-MET*-mutated patients.

1. Introduction

Small Cell Lung Cancer (SCLC) accounts for approximately 13–15% of new lung cancers each year [1]. SCLC is strongly associated with smoking and is characterized by an extremely aggressive behavior with early systemic spreading. Therefore, SCLC is frequently diagnosed in advanced stage (Extensive Disease, ED) and palliative chemotherapy is the only therapeutic approach [2]. Despite brilliant response to first-line therapy, SCLC ultimately relapses and shows very low sensitivity to second-line treatment. Overall, the prognosis is poor with a median survival of approximately 16–24 months for patients with limited disease (LD) treated with chemo-radiotherapy and 7–12 months for patients diagnosed with ED [3].

Understanding the genetic basis of SCLC is of primary importance to guide the development of effective drugs [4,5]. No targeted agent has yet demonstrated any efficacy in SCLC therapy; most trials, however, did not select patients based on specific genetic changes [4,5]. In analogy with other lung cancer models, the identification of drugable gene mutations is an emerging issue in SCLC management.

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We investigated the mutational status of several oncogenes (*EGFR, c-MET, BRAF, KRAS, PDGFRa, c-KIT*) for which target drugs are available in a retrospective series of SCLC patients from 2 Italian institutions. Clinical data, outcomes and immunohistochemical expression of TTF-1, chromogranin, CD56, p63, synaptophysin and bcl-2 were also collected to investigate possible correlations between mutational status and clinical-pathological features.

2. Materials and methods

2.1. Patients

One hundred and thirteen cases of primary pulmonary SCLC diagnosed between 2001 and 2010 were collected from the files of the Units of Surgical Pathology of Modena and Parma. Samples were selected based on quantity and quality of tissue available for genetic analysis. Patients with other known primary tumors were excluded. Two pathologists (GR, LG) reviewed all original histological slides; tumors were reclassified according to the criteria set by the 2004-WHO lung tumors classification [6] reaching uniform consensus in all cases.

There were 108 (96%) bronchial/trans-bronchial biopsies and 5 (4%) surgical resection specimens. All samples were routinely fixed in 10% buffered formalin for at least 12 hours and no more than 48 hours and then embedded in paraffin blocks. Clinical data were collected in all cases from pathological reports, clinical charts, referring physicians or directly from the patient's families. The recorded data were: age, sex, smoking history, response to therapy and survival. Staging was evaluated according to the Veterans Affairs classification system (limited and extensive disease).

As to with smoking habit, patients were subdivided in never-smokers (lifetime exposure of less than 100 cigarettes), former smokers (smoking cessation more than 1 year before the diagnosis) and current active smokers.

Tumor response to therapy was assessed by imaging studies according to the Response Evaluation Criteria in Solid Tumors (RECIST) criteria [7]. Time To Progression (TTP) was defined as the time from diagnosis to the time of the first disease progression. Overall Survival (OS) was defined as the time from diagnosis until death from any cause. The investigators were blinded to patient outcomes.

2.2. Immunohistochemical (IHC) stains

Immunostains were performed on an automated immunostainer (Benchmark, Ventana, Tucson, AZ) using the following antibodies (Ventana): TTF-1 (clone 8G7G3/1), chromogranin A (clone LK2H10), CD56/NCAM (clone 123C3D5), p63 (clone 4A4), synaptophysin (polyclonal) and bcl2 (clone bcl2) according to the manufacturer's instructions. For diagnostic purposes on crushed biopsies, also Ki67 (clone MIB1, Ventana) was performed in selected cases.

Negative and positive controls were included in each batch. For each antibody, the percentage of positive cells and the intensity of staining (0: negative; 1+: weak; 2+: moderate; 3+: strong) were recorded. A tumor was considered positive when at least 10% of the neoplastic cells reacted with an intensity of 2+ or greater on the relevant subcellular localization.

2.3. DNA extraction and mutational analysis

Five μm-thick, hematoxylin–eosin-stained sections were applied on noncover-slipped slides. Tumor cells were microdissected under an inverted microscope using a sterile blade and directly transferred to a tube containing a lysis buffer [2 mg/ml proteinase K in 50 mM Tris (pH 8.5), 1 mM EDTA, 0.5% Tween 20]. Samples were incubated overnight at 56 °C; proteinase K was heat-inactivated at 95 °C for 10 min. PCR was performed in a final volume of 20 μl containing 2.0 μl DNA, 2 μl of commercial PCR buffer (Applied Biosystems, Foster City, CA), 1.0–2.0 mM of $MgCl_2$, 200 mM of each dNTP, 20 pmol of each primer, and 3 units of AmpliTaq Gold polymerase (Applied Biosystems). PCR was carried out on Uno II Thermoblock (Biometra, Gottingen, Germany). Initial denaturation at 95 °C for 10 min was followed by 41 cycles and a final extension step (10 min at 72 °C). The cycles included denaturation at 95 °C for 1 min, annealing at 55–66 °C for 1 min, and extension at 72 °C for 2 min.

The amplified DNA was electrophoresed on 2% agarose gel to check the reaction products and then purified using the MinElute PCR purification Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions.

PCR products were sequenced in both directions with the ABI Prism BigDye Terminator v1.1 Cycle Sequencing kit (Applied Biosystems) and run on an ABI Prism 310 automatic sequencer (Applied Biosystems). Sequencing data were analyzed with *Sequencing Analysis* 5.2 Software (Applied Biosystem). The primers used to amplify *c-KIT* exons 9, 11, 13, 14 and 17, *PDGFRa* exon 12, 14 and 18, *c-MET* exons 14, 17, 18 and 19, *EGFR* exons 18, 19, 20 and 21, *KRAS* exon 2 and *BRAF* exon 15 are listed in supplemental table.

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.lungcan.2014.10.002.

2.4. Statistical analysis

Correlations between categorical variables were calculated using chi-square and Fisher's exact tests. Univariate analysis was performed by the Pearson correlation coefficient using the SPSS package, version 13.0 (Chicago, Inc., USA). Statistical significance was for P values <0.05; all tests were two-sided.

3. Results

3.1. Patients' characteristics

The main clinical features of the study base are summarized in Table 1. Median age was 69 years (range 32–86), patients were mainly male (75.2%) and affected by ED (65.5%); 85 (75.2%) were current smokers, 25 (22.1%) were former-smokers and only 3 patients (2.7%) were never-smokers. One hundred and seven patients were treated with chemotherapy, of whom 45 (39.8%) with cisplatin-etoposide, 60 (53.1%) with carboplatin-etoposide and 2 (1.8%) with different regimens (1 patient received etoposide alone and 1 patient was treated with cisplatin for 2 cycles switched to carboplatin for the 3rd cycle). Six patients did not receive any treatment. Thirty-five (31%) patients had thoracic radiotherapy as part of treatment.

Three and six months Response Rate (RR) were 58.4% (66 patients) and 32.7% (37 patients), respectively. Median TTP of the entire series was 6 months (95%CI 5–7 months), while median OS reached 11 months (95%CI 10–12 months).

3.2. Immunohistochemical (IHC) and molecular analysis

Results of IHC analysis are shown in Table 2. Seventy out of 95 (73.7%) and 78 out of 97 (80%) patients immunostained for TTF-1 and chromogranin A, respectively. CD56, synaptophysin and bcl-2 were expressed in 102 out of 107 (95%), 61 out of 107 (57%) and 80 out of 82 (97.5%), respectively. All tested tumors (83) were negative for p63.

Tissue samples were analyzed for mutations of the *EGFR, c-MET, BRAF, KRAS, PDGFRa* and *c-KIT* genes. Results are summarized in