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Original Contributions

Non-small cell lung carcinomas with *CTNNB1* (beta-catenin) mutations: A clinicopathological study of 26 cases

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ABSTRACT

Beta-catenin, encoded by the *CTNNB1* gene, plays an important role in cell proliferation. Mutations of *CTNNB1* are oncogenic in several tumor types and are often associated with a nuclear abnormal expression. However, such mutations have only rarely been reported in non-small cell lung carcinomas and their clinical signification is not well described.

Our study was conducted on 26 *CTNNB1*-mutated non-small cell lung carcinomas. Tumors were routinely tested by next generation sequencing for mutations in exon 3 of *CTNNB1* gene. Twenty three cases were from a series of 925 tumors (2.48%). The hospital files and pathological data, from surgical samples (n = 16), small biopsies (n = 5) and trans-bronchial fine needle aspirations (n = 5), were reviewed. Immunohistochemistry was performed with an anti-beta-catenin antibody.

There were 10 female and 16 male patients aged 52 to 83. Eleven of 25 patients were no-smoking or light smokers. Three cases were diagnosed while under treatment with *EGFR* tyrosine kinase inhibitor. There were 25 adenocarcinomas and 1 squamous cell carcinoma. Most adenocarcinomas had a papillary component and were TTF1-positive. One case was a well-differentiated fetal adenocarcinoma. Eleven cases (42%) with *CTNNB1* mutations showed associated *EGFR* mutations. The frequency of *CTNNB1* mutations was higher among *EGFR* mutated carcinomas. Immunohistochemistry showed heterogeneous nuclear or cytoplasmic abnormal expression.

Our study shows that *CTNNB1* mutations mostly occur in TTF1-positive adenocarcinomas with a papillary pattern. These mutations are often associated with *EGFR* mutations and possibly interfer in the mechanism of resistance to tyrosine kinase inhibitors. Our experience suggests that immuno-histochemistry cannot be used for screening.

1. Introduction

Beta-catenin, encoded by the *CTNNB1* gene, is important for cell adhesion and is mainly present in the cell membranes [1]. Beta-catenin also plays an important role in a signaling pathway of progenitor cell proliferation and differentiation. Mutations of *CTNNB1* are oncogenic in several tumor types [1]. These mutations result in the stabilization of the protein leading to cytoplasmic and nuclear accumulation [2]. *CTNNB1* mutations are rare in non-small cell lung carcinoma (NSCLC). In 2 large series, *CTNNB1* mutations were only observed in 11 of 546 [3] and in 10 of 425 NSCLC patients [4]. A higher frequency of *CTNNB1*mutations has been reported in pulmonary adenocarcinoma of fetal lung type, which is a rare form [5]. The clinical signification of *CTNNB1* mutations in NSCLC is not well described. Assessment of *CTNNB1* mutations in NSCLC is not recommended in current routine practice. Other biomarkers, such as *EGFR*, *KRAS*, *ALK* or PDL1, have more clinical interest [6]. However, molecular technics with multiplex panels, which can be used for different tumor types and not only for

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NSCLC, enable to have data related to many genes including *CTNNB1* [3]. We retrospectively reviewed a series of NSCLC, which were found to be *CTNNB1* mutated by routine genetic analysis.

2. Materials and methods

Our study was conducted on 26 *CTNNB1*-mutated non-small cell lung carcinomas. Twenty three cases were from a series of consecutive and contributive analyses performed on 925 tumors (frequency: 2.48%), between January 2017 and April 2019. The 3 other cases dated from before this series. The 925 consecutive cases included 671 adenocarcinomas, 114 squamous cell carcinomas and 140 other non-small cell carcinomas.

Molecular analyses were routinely performed by Next-Generation Sequencing on PGM technology (Thermofisher), with the kit Sentosa® SQ NSCLC Panel (Vela Diagnotics) covering main mutations in 11 genes or with the kit Sentosa® SQ Onco Key Panel (Vela Diagnostics) covering main mutations in exons of 73 genes, both including exon 3 of *CTNNB1*.

The hospital files of the 26 patients and the pathological data were reviewed. Samples for genetic analysis were from surgical resections ($n = 13$), open pleural biopsies ($n = 3$), trans thoracic needle biopsies ($n = 3$), bronchial biopsies ($n = 2$) and trans-bronchial fine needle aspirations ($n = 5$). Tumor samples from surgical specimens were also available for review in 2 cases of trans-thoracic needle biopsy and 1 case of trans-bronchial fine needle aspiration. Tumor stage was assessed at the time of β-catenin mutation diagnosis. Immunohistochemistry was performed with an anti-beta-catenin antibody (#610154, BD Biosciences).

Statistical analysis was performed by Fisher's exact test.

3. Results

Main clinico-pathologic characteristics are summarized in Table 1.

3.1. Clinical and epidemiologic data

Ten patients had a history of lung carcinoma, treated 1 to 13 years (mean 4.6) previously. These 10 cases could represent progressions, recurrences or new cancers. The previous lung carcinomas had been treated by surgery alone ($n = 2$), surgery and chemotherapy ($n = 3$), chemotherapy and radiotherapy ($n = 1$), or targeted therapies ($n = 4$). Targeted therapies included *EGFR* tyrosine kinase inhibitors when *EGFR* mutation was present ($n = 3$) or trastuzumab in one case with *HER2* mutation. Unfortunately, the *CTNNB1* mutational status of the initial tumors was unknown.

In our series, the frequency of CTNNB1 mutation was 7.40% (10/135) among carcinomas with EGFR mutation and only 1.65% (13/790) in absence of EGFR mutation ($p = 0.00099$).

3.2. Pathologic features

Most cases (25/26) were adenocarcinomas. Most morphological variants were observed (lepidic, acinar, solid, papillary, mucinous). However among reviewed surgical specimens, 16/19 (84%) showed a predominant ($n = 12$) or minority ($n = 4$) papillary component. One case showed features of low grade fetal adenocarcinoma (Fig. 1). Most adenocarcinomas were TTF1-positive (23/26). The only squamous cell carcinoma (Fig. 2) was diagnosed on surgical resection specimen; any associated adenocarcinomatous component was not observed and immuno-histochemistry showed TTF1 negativity and diffuse p63 positivity. Immunohistochemistry with anti-PDL1 antibody showed positivity in 0%, 1 to 49% or 50% or more of the tumor cells, in 16, 7 and 2 of the 25 tested cases, respectively. There was no ALK positive case (0/23).

Immunohistochemistry with anti-beta-catenin antibody showed some cytoplasmic and nuclear staining, in addition to a constant normal strong membranous expression. However the abnormal cytoplasmic and nuclear staining was heterogeneous between cases and within each case (Fig. 3).

Table 1
Main clinico-pathologic characteristics of the 26 lung carcinomas with *CTNNB1* mutation.

Age (years)	52–83 (mean = 65.0)
Sex (F/M)	10 F/16 M
Smoking habit	Smokers (> 10 PY): 14/25 (56%)
Tumor stage	I: 5 (19%) II:4 (15%) III:5 (19%) IV:12 (46%)
Histology	Adenocarcinoma: 25 (96%) Squamous cell carcinoma: 1 (4%)

3.3. Genetics

The most frequent *CTNNB1* exon 3 mutations were S37F ($n = 8$, 30.8%) and S45P ($n = 5$, 19.2%). Other were S33C ($n = 3$), G34R ($n = 2$), S37C ($n = 2$), D32H, D32V, G34V, S33F, S33Y and T41A (1 case of each). The allele frequency of *CTNNB1* mutations ranged from 1 to 35% (mean = 13 ± 11).

Eleven of the 26 cases (42%) with *CTNNB1* mutations showed associated *EGFR* mutations including exon 19 deletion ($n = 7$) and L858R (exon 21) ($n = 3$). One exon 19 deletion was also associated with a G719A (exon 18) mutation and one L858R mutation was also associated with a T790M (exon 20) mutation. One case showed both G719 (exon 18) and S768I (exon 20) mutations. In most cases (8/10), the allele frequency of *CTNNB1* mutations was lower or equal to the allele frequency of *EGFR* mutations. Six of the 15 *EGFR*-wild type cases showed KRAS ($n = 3$), BRAF ($n = 1$), HER2 ($n = 1$) or STK11 ($n = 1$) mutations.

4. Discussion

Our series confirms that *CTNNB1* mutations are rare in lung carcinomas. One could think that these mutations were only “bystanders” in a tobacco-related cancer with high mutation burden. However we observed that these mutations do not seem to occur randomly. Only less of half of our cases occurred in non- or light smokers. However *CTNNB1* mutations were reported to be significantly more frequent in ex-light-smokers [4] and in another series these mutations were mostly (8/11) observed in never smokers [3]. In our series, the rate of *CTNNB1* mutations were 4.5 time more frequent in *EGFR*-mutated carcinomas than in *EGFR*-wild type tumors. Similarly, *CTNNB1* oncogenic mutations had been reported to be more frequent in *EGFR*-mutation-positive patients (5.3%, 60/1122) than in *EGFR*-mutation-negative patients (1.8%, 17/944, $q = 2.0 \times 10^{-4}$) [7]. Conversely, *EGFR* co-mutations are frequent in *CTNNB1*-mutated NSCC: 42% (11/26) in our series, 73% (8/11) in a American series [3] and 20% (2/10) in a Finnish series [4], It would be interesting to know if it were also the case in Asian countries where *EGFR* mutations are much more common.

Three of our cases occurred in patients under *EGFR* tyrosine kinase inhibitor. *CTNNB1* mutations might influence the efficacy of targeted treatment, leading to primary or secondary resistance [4]. *CTNNB1* mutations were more frequently found in EGFR p.Thr790Met-positive (33/440) than in EGFR p.Thr790Met-negative patients (27/682, $q = 0.12$) [7]. However, in a series of 21 NSCLC with *EGFR* mutation and receiving first-line erlotinib treatment, 2 cases harboring concomitant *CTNNB1* mutations are reported partially responding [8]. In a series of 132 patients with *EGFR* mutations and treated with gefitinib, 7 (5.3%) harbored a concomitant *CTNNB1* mutation and only 1/7 was gefitinib-resistant [9]. Similarly 2 patients out of 17 (11,8%) with *EGFR* and *CTNNB1* comutations were both good responders to gefitinib in an

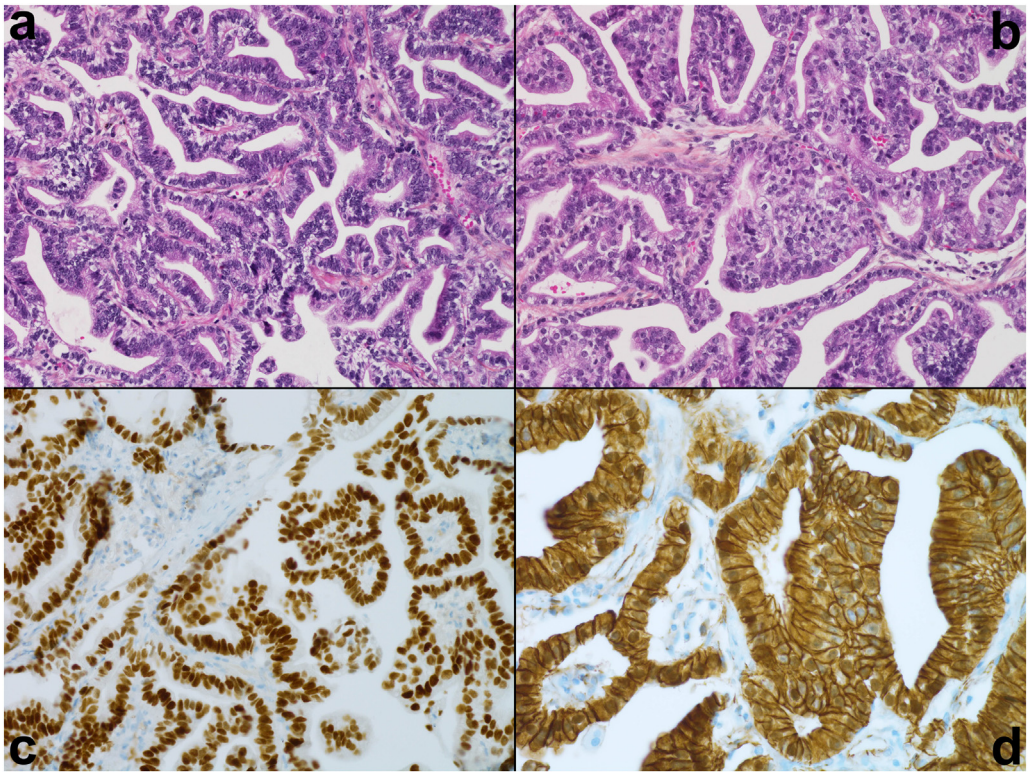

Fig. 1. *CTNNB1* mutated lung carcinoma showing features of fetal type adenocarcinoma. Complex glandular structures with clear pseudo-stratified epithelium, a scant stroma (a) and morule formation (b). TTF1 expression (c). Nuclear and cytoplasmic anbnormal expression of beta-catenin associated with normal membrane expression (d).

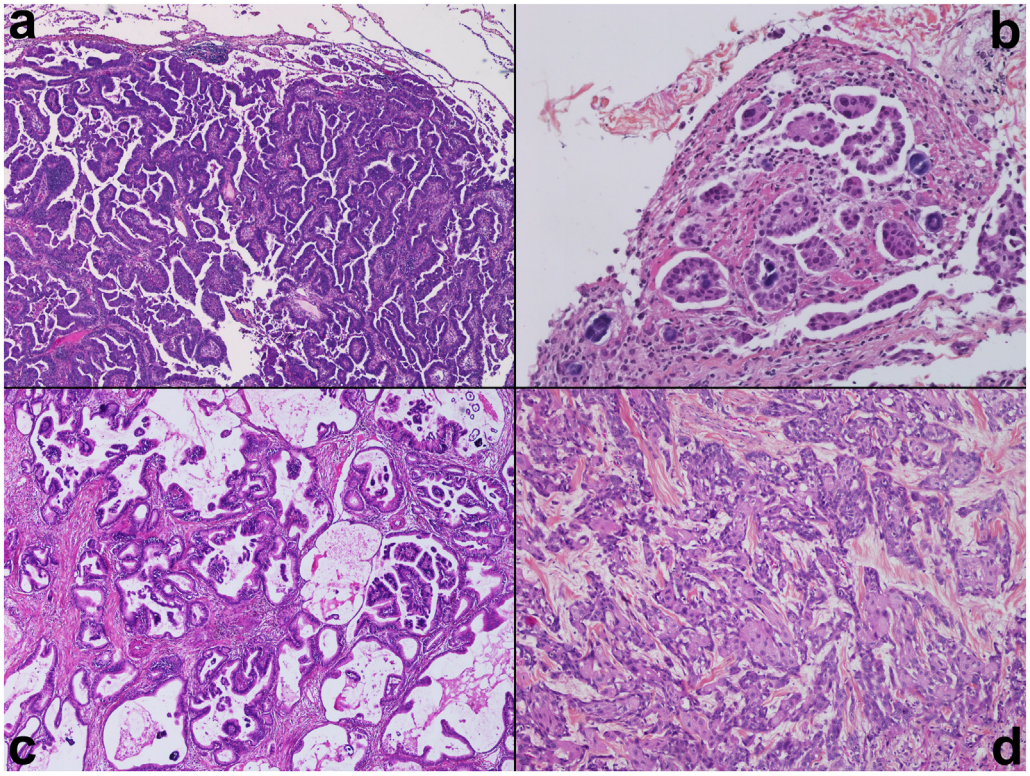

Fig. 2. Morphological heterogeneity of *CTNNB1* mutated lung carcinomas: Papillary architecture was often obvious (a). It was suggested in one case with calcospherites on a pleural biopsy (b). One case was mucinous (c). One case was a squamous cell carcinoma (d).

Italian series [10].

Among lung tumors, *CTNNB1* mutations have only previously been reported in adenocarcinomas [3,4]. Our case of squamous cell carcinoma was unexpected. It is not possible to rule out the possibility of an adenosquamous carcinoma with undetectable adenocarcinomatous component, even if the diagnosis was made on a surgical resection specimen.

Beta-catenin abnormal nuclear expression may be diagnostic of tumor type and of *CTNNB1* mutations in sporadic desmoid tumors [2]. However, immuno-histochemistry has less diagnostic value in other

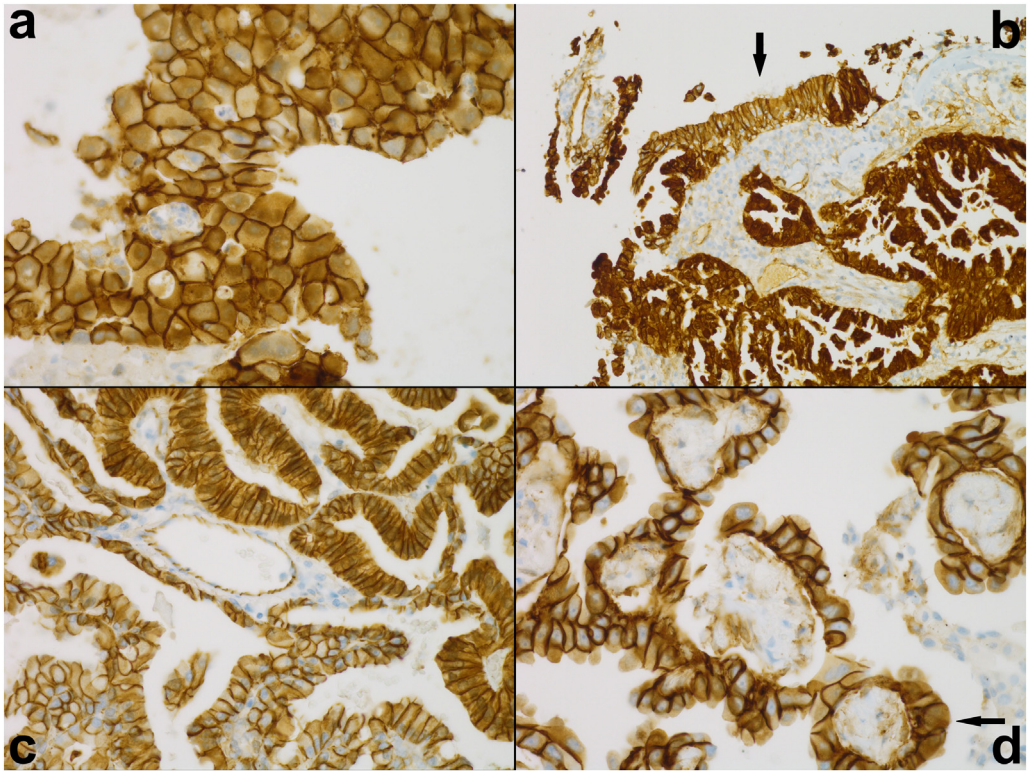

Fig. 3. Beta-catenin expression in *CTNNB1* mutated lung carcinomas: Cytoplasmic and nuclear expression in addition to strong normal membrane expression on a transbronchial needle aspiration specimen (a). Strong cytoplasmic staining contrasting with only membrane expression by normal epithelium (→) on a bronchial biopsy (b). Staining heterogeneity in a case with nuclear/cytoplasmic expression restricted to the right upper half of the picture (c). Case with only focal nuclear/cytoplasmic expression (→)(d).

contexts and for tumors that are less associated with these mutations, such as endometrial carcinomas [11] or salivary gland tumors [12]. Diagnostic of *CTNNB1* mutations may require the use of several different antibodies, as shown in colorectal carcinomas [13]. The heterogeneity of staining that we observed with anti-beta-catenin antibody could be related to the heterogeneity of *CTNNB1* mutations among tumor cells. This heterogeneity and the low rate of mutated cases make immunohistochemistry unfit for screening.

Otherwise, regardless of *CTNNB1* mutations, beta-catenin expression has been studied as a prognostic factor in non-small cell lung carcinomas, with discordant results [14]. Since beta-catenin is also involved in immuno-suppression (reduction of T-cells infiltrates) in the tumor microenvironment [1,15], its role in sensibility to immunotherapy has also been explored. Beta-catenin expression in adrenocortical carcinoma has been reported to be negatively correlated with PD-L1 expression [15]. Our series was too small to draw any conclusion on these matters.

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