論 文 内 容 要 旨

Intense Expression of EGFR L858R Characterizes the Micropapillary Component and L858R Is Associated with the Risk of Recurrence in pN0M0Lung Adenocarcinoma with the Micropapillary Component

（微小乳頭状構造(micropapillary component)を含む病理病期N0M0 肺腺癌では、上皮成長因子受容体(EGFR)のL858R 変異発現がmicropapillary component を特徴づけるとともに再発リスクとなる）

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Background

Micropapillary (MP) predominant adenocarcinoma is one of adenocarcinoma phenotypes and known as highly malignant potential type. Although the incidence of MP predominant adenocarcinoma is low (0.85–16.2%), adenocarcinoma containing MP pattern as its non-dominant component (including > 1% MP component) is commonly detected (10.7–65.8%) and is also associated with poor prognosis. MP components often coexist with papillary (PaP) components or accompany PaP predominant adenocarcinoma. These 2 subtypes are distinguished based on the morphology. The clinicopathological or genetic characteristics of patients with MP component have been evaluated. The specific genetic variants of the MP component have not been reported. It also remains unknown whether MP component and PaP component vary genetically; if they vary, how the difference is related to the malignant potential on patients with MP component. We comprehensively compared mRNA expression between the MP and PaP components in the same patient and explored to identify the genetic traits responsible for the poorer prognosis in patients with the MP component.

Materials and methods

Primary lung adenocarcinoma patients with > 20% MP component and PaP component were selected for mRNA expression analysis. MP and PaP components were separately captured from formalin-fixed paraffin embedded tissue by laser microdissection and mRNA was extracted from 10 patients. RNA of adequate quality (> 100 ng and ≥ 50% DV200 after purification) could be extracted from both the MP and PaP components in three patients. Comprehensive mRNA expressions of somatic variants were compared between MP and PaP components of each patient using next-generation sequencing (NGS). NGS was performed with a HiSeq 2500 (Illumina, San Diego, CA, USA). Sequence reads were processed and mapped to a human genome reference sequence (hg19) with TopHat v. 2.0.14. Mutations were detected with GATK v. 3.6 according to GATK Best Practices (GATK. https://software.broadinstitute.org/gatk/). Somatic variants were detected with varscan v. 2.4.2 at the default setting. Fisher's exact test was used to determine the significance level of allele frequency. Detected mutations were annotated with snpEff v. 4.2 (http://snpeff.sourceforge.net/). Somatic variants were considered cancer-related if they were detected in COSMIC (https://cancer.sanger.ac.uk/cosmic). The protein expression of NGS-detected genetic variant was validated by immunohistochemistry (IHC); The immunoreactivity levels (staining intensity and the proportion of positive cells) of the MP and PaP components were compared between MP and PaP component. Furthermore, prognostic impact of NGS-detected variant was evaluated in 288 invasive (excluding minimally invasive adenocarcinoma) pN0M0 adenocarcinoma patients using overall

survival (OS) and recurrence-free survival (RFS).

Results

The numbers of genetic variants detected in the MP and PaP components of 3 patients by NGS were 93077-179002 and 83427-186031, respectively. Among them, 9-23 pathogenic or cancer-related variants were detected in each patient. Cancer-related variants were more frequently detected in the MP component of each patient and one variant in EGFR (L858R) was expressed at significantly higher levels in two patients (allele frequency = 0.485 vs 0.155 and 1.000 vs 0.526, respectively $P < 0.001$ in both). IHC was performed to validate the NGS results in 27 MP-positive patients harboring L858R. All 27 patients had the PaP component and 17 cases presented with heterogenous intratumor immunoreactivity. IHC score for L858R protein was significantly higher in MP components ($P < 0.001$). Of the 288 invasive pN0M0 adenocarcinoma patients, the MP-positive patients were more often included among the PaP-predominant patients. MP-positive patients showed significantly higher frequencies of pleural invasion, lymphatic and vascular invasion, L858R mutation, and recurrence than MP-negative patients. The five-year RFS were 87.6% and 75.6% in the MP-negative and MP-positive patients, respectively. The five-year RFS for the MP-/L858R-, MP-/ L858R+, MP+/ L858R-, and MP+ /L858R+ cases were 89.4%, 84.2%, 84.6%, and 46.9%, respectively. MP-positive patients showed significantly worse RFS than MP-negative patients (MP- vs. MP+: hazard ratio (HR) = 2.074, 95% confidence interval (CI) = 1.116–4.616, $P = 0.024$). There was no significant difference between the MP-negative patients without the L858R mutation and those harboring L858R in terms of RFS (MP-/L858R- vs. MP-/L858R+: HR = 1.061, 95% CI = 0.380–2.968, $P = 0.909$). In contrast, MP+/L858R+ patients presented with significantly worse RFS than MP+/L858R- patients (MP+/L858R- vs. MP+/L858R+: HR = 3.004, 95% CI = 1.306–9.132, $P = 0.012$). Multivariate analysis in MP-positive patients revealed positive L858R status was associated with poorer RFS (HR = 2.976, 95% CI = 1.190–7.442; $P = 0.020$). The five-year OS were 93.1% and 85.4% for the MP-negative and MP-positive patients, respectively. The five-year OS for the MP-/L858R-, MP-/L858R+, MP+/L858R-, and MP+/L858R+ were 92.8%, 95.1%, 87.0%, and 81.8%, respectively. There were no significant differences in OS among any of the variants.

Conclusions

Micropapillary component of lung adenocarcinoma was genetically characterized by *EGFR* L858R, which is also related to malignant potential. *EGFR* L858R is more frequently harbored in MP-positive adenocarcinoma patients than in MP-negative adenocarcinoma patients. Intense expression of L858R was suggested in MP component and MP-positive patients harboring L858R are at comparatively higher risk of recurrence in pN0M0 lung adenocarcinoma.