ORIGINAL ARTICLE

Synergistic Activation upon *MET* and *ALK* Coamplification Sustains Targeted Therapy in Sarcomatoid Carcinoma, a Deadly Subtype of Lung Cancer

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ABSTRACT

Introduction: Genetic alterations suitable for targeted therapy are poorly known issues in pulmonary sarcomatoid carcinoma (PSC), an uncommon and life-threatening family of non–small cell lung cancers.

Methods: Ninety-eight PSCs were assessed for MNNG HOS Transforming gene (*MET*) and anaplastic lymphoma receptor tyrosine kinase gene (*ALK*) status by fluorescence in situ hybridization (FISH) and for relevant protein expression by immunohistochemical analysis, also taking advantage of phosphorylated (p-) antibodies. Moreover, levels of *ALK* and *MET* mRNA were also determined by real-time polymerase chain reaction and Western blot analysis for downstream activation pathways involving p-MET, p–protein kinase B, p–mitogen-activated protein kinase, p-SRC proto-oncogene tyrosine-protein kinase, and p–focal adhesion kinase (p-FAK).

Results: *MET* amplification was detected by FISH in 25 of 98 PSCs (25.6%) and *ALK* amplification (but not the relevant rearrangement) was found in 16 of 98 (16.3%), with all *ALK*-amplified tumors also showing *MET* amplification ($p < 0.0001$). Nine PSCs, however, showed *MET* amplification without any *ALK* gene alteration. ALK protein expression was always lacking, whereas MET and p-MET were confined to the relevant amplified tumors only. Increased levels of *ALK* and *MET* mRNA were detectable in tumors with no

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direct relationship between mRNA content, protein expression, or alterations detected by FISH. Western blot assays showed complete activation of downstream signal pathways up to p-SRC proto-oncogene tyrosine-protein kinase, and p–focal adhesion kinase recruitment in *MET* and *ALK*–coamplified tumors only, whereas isolated *MET* amplification, *MET* and *ALK* borderline amplification (5%–10% of tumor cells with $\geq$15 copies of the relevant gene), or negative tumors showing eusomy or chromosome polysomy were confined to p–mitogen-activated protein kinase, p–protein kinase B, and/or p-MET activation. Multivariate survival analysis pushed a higher percentage of *MET* altered cells or a higher value of *MET* copy gain per cell to marginally emerge for overall survival ($p = 0.140$) and disease-free survival ($p = 0.060$), respectively.

Conclusions: *ALK* and *MET* seemed to act as synergistic, nonrandom coactivators of downstream signal when coamplified in a subset of patients with PSC, thus likely suggesting a combined mechanism of oncogene addiction. These alterations could be a suitable target for therapy based on specific inhibitors.

Keywords: MET; ALK; FISH; Sarcomatoid carcinoma; Lung; Survival

Introduction

Pulmonary sarcomatoid carcinoma (PSC) makes up a rare (2%–3%) but deadly family of non–small cell lung carcinomas (NSCLCs) encompassing pleomorphic carcinoma (PLC) (the most frequent), spindle cell carcinoma (SpCC), giant cell carcinoma (GCC), carcinosarcoma (CS), and pulmonary blastoma (PB).[1] PSCs are deemed to be monoclonal tumor growths, in which a stable and huge epithelial-mesenchymal transition (EMT) takes place, causing sarcoma-like and/or sarcoma elements to arise.[1–8] As the sensitivity of PSC to current medical manipulations with platinum-based doublets, sarcoma-specific regimens, or radiotherapy is disappointing, reversing the EMT or targeting specific oncogene addiction mechanisms could be suitable therapy options for so life-threatening a tumor family.[9–12] However, the lack of specific PSC-oriented clinical trials, the inherent rarity of these tumors along with trouble in their diagnostic recognition, and the scarce understanding of the biological mechanisms underlying the development and progression of these tumors explain their current unsuitability for a more effective clinical management beyond surgical excision.[1,2,11,12] Therefore, additional genetic testing with a variety of experimental tools could allow new targets of personalized intervention to be established along with the relevant selection criteria to identify patients. Taking into account the enormous prevalence and the social relevance of lung cancer in the human population worldwide,[13] even rare tumors such as PSC are clinically meaningful at the level of individual patients.

A nonrandom amplification but not structural rearrangement of the anaplastic lymphoma receptor tyrosine kinase gene (*ALK*) gene has recently been documented in approximately 18% of PSCs by means of fluorescence in situ hybridization (FISH) analysis.[14] As no ALK protein accumulation was consistently observed by sensitive immunohistochemical (IHC) methods, another synergistic gene driver mechanism, such as the MNNG HOS Transforming gene (*MET*) gene, which is recurrently altered in a subset of patients with PSC[14,15] with significant functional differences with lung adenocarcinoma, was hypothesized to be in charge of the development and maintenance of these tumors.[16–19] More recently, it has been published that mutational events of *MET* leading to exon 14 skipping are potentially targetable events in PSC,[11] but the relevance of this mutation has been diversely reported on.[12] Most important, the role of *MET* amplification and its functional interplay with *ALK* alterations still remains an unresolved issue.

Beyond functioning as a driver gene in the development of PSC and an adverse prognostic factor in lung cancer,[20] *MET* is also recruited as a primary or secondary mechanism of resistance to tyrosine kinase inhibitor treatment in epidermal growth factor receptor gene (*EGFR*)-mutated lung adenocarcinoma,[21–24] whereas de novo *MET* amplification is restricted to approximately 3% to 5% of chemonaive patients with adenocarcinoma.[16,23,25]

This study was aimed at evaluating the status of *MET* and the relative interplay with *ALK* in a consecutive series of PSCs. It is the largest study thus far to be assessed for either biomarker by using several experimental approaches, such as FISH, IHC analysis, real-time polymerase chain reaction (PCR), and functional activation upon Western blot. The main goal was to offer a biological rationale for the functional utilization of *MET* as a suitable target of therapy for these life-threatening tumors.

Patients and Methods

Overall Design of the Study

Cases were screened for *ALK* and *MET* gene status abnormalities by means of FISH and for the relevant expression of either total or phosphorylated (p-) protein by using enzymatic IHC analysis. Subsequently, the functional meaning of the diverse gene alterations was further untangled by using real-time PCR to evaluate the mRNA levels and Western blot analysis to decipher the relevant