Rapid Symptomatic Improvement in Gefitinib-Treated Patients with EGFR-Mutated Lung Cancer: Possible Role of Downregulation of Inflammatory Molecules?

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The tyrosine kinase inhibitors (TKI) of the epidermal growth factor receptor (EGFR), gefitinib (IressaTM, AstraZeneca, Wedel, Germany) or erlotinib (Tarceva[®], Roche Pharma AG, Grenzach-Whylen, Germany), induce reduction of tumor size in virtually all patients with advanced non-small cell lung cancer (NSCLC), whose tumors harbor a sensitizing mutation in the EGFR gene [1, 2]. It has been repeatedly noticed that while the shrinking of the tumor occurs in a gradual manner, the majority of responders demonstrate dramatic symptomatic relief within the very first days or even hours of treatment [3]. This immediate improvement cannot be explained simply by reduction of the tumor mass, therefore other mechanisms have to be considered. Peritumoral inflammation may significantly contribute to the discomfort experienced by cancer patients. In vitro studies demonstrate that EGFR activation is associated with induction of a variety of proinflammatory molecules (e.g., IL6, IL8, ICAM1), and this effect is reversed upon administration of EGFR-specific inhibitors [4–6]. Here we present the results of a comparative analysis of pre- and post-treatment tumor samples from a patient who was diagnosed with EGFR-mutated NSCLC and received gefitinib before surgery.

A 44-year-old female patient was referred to the N.N. Petrov Institute of Oncology with complaints of hemoptysis, fatigue, incapacitating edema of the lower extremities, arthralgia and fever (ECOG status 2–3). Standard X-ray examination and computed tomography (CT) scan of her chest revealed a tumor in the lower lobe of the right lung accompanied by the involvement of hilar and bifurcational lymph nodes (stage T2N2M0/IIIA); no extrapulmonary lesions were identified upon appropriate diagnostic procedures. The affected tissue

was obtained by bronchoscopy, and the morphological analysis classified the tumor as adenocarcinoma. A subsequent EGFR mutation test revealed a canonical TKI-sensitizing deletion located within exon 19 of the gene. Given the poor performance status of the patient, the extensive local tumor spread and the presence of a highly predictive EGFR mutation, immediate surgical intervention was considered unreasonable. Instead, neoadjuvant therapy with gefitinib (250 mg daily) was initiated. In addition, hemostatic treatment with etamsylate (250 mg by intramuscular injection, 3 times daily) was performed for 3 days. The prescribed therapy led to rapid symptomatic relief: by day 4, the ECOG status reached 1, and no signs of hemoptysis or paraneoplastic edema were observed. X-ray and CT examinations were repeated on day 12 of gefitinib therapy. A dramatic reduction of the primary tumor and hilar lymph nodes as well as disappearance of the bifurcational lesions were detected (fig. 1); given the significant downstaging of the disease and good performance status of the patient, a right pneumonectomy with systematic ipsilateral mediastinal lymph node dissection was performed 2 days later.

Both pre- and post-treatment tissue samples contained a sufficient number of neoplastic cells, therefore it was possible to microdissect tumor tissues, isolate RNA, and perform reverse transcription polymerase chain reaction (RT-PCR) expression analysis of a few candidate genes. In addition to the above mentioned EGFR-inducible inflammatory molecules (IL6, IL8, ICAM1), we also considered the TNF-alpha and IL1-beta genes. Both these cytokines contribute to the mobilization of immune cells. TNF-alpha is involved in the formation of peritumoral pleural effusion [7]. A specific haplotype of the IL1-beta was shown to be associated with increased gene expression and elevated lung cancer risk [8]. As a control, we deliberately included in the study 2 genes, which are not related to inflammatory reactions, DPD and ERCC1.

Quantitative real-time PCR was performed as described in [9]. mRNA expression analysis revealed that the TNF-alpha, IL1-beta and IL8 genes were evidently downregulated during gefitinib therapy (table 1, fig. 1). The IL6 transcript was not seen at all in the pre-treatment sample and was only barely detectable after exposure to the EGFR inhibitor. The expres-

sion status was not changed significantly for ICAM1, DPD and ERCC1.

This study confirmed that EGFR inhibition may reduce the activity of several inflammation-related genes in vivo. This effect may contribute to the rapid symptomatic improvement observed upon EGFR TKI administration. It would be highly desirable to compare pre- and post-treatment tumor samples by RNA array profiling. Unfortunately, currently available transcriptomic technologies are not yet fully adjusted to dealing with micro-amounts of RNA and/or archived tissue samples.

Fig. 1. Downregulation of inflammatory molecules in the EGFR-mutated lung adenocarcinoma treated by gefitinib.

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Disclosure Statement

The authors declare no conflict of interest.

Table 1. Real-time RT-PCR analysis of an EGFR-mutated lung cancer sample before and after gefitinib therapy

Gene	PCR primers	Analyzed/referee mRNA ratio		Before/after therapy expression ratio
		before therapy	after therapy	
TNFA	CACCACGCTCTTCTGCCT TGACTGCCTGGGCCAGAG	0.62	0.16	3.88
IL1B	GAGAATGACCTGAGCACCT CGTGCAGTTCAGTGATCGTA	0.20	0.07	2.86
IL6	CTGAGAAAGGAGACATGTAAC CACCAGGCAAGTCTCCTCAT	0.00	0.01	–
IL8	GACATACTCCAAACCTTTCCA GACAGAGCTCTCTTCCATCA	0.70	0.14	5.00
ICAM1	GTGCTATTCAAACCTGCCCTG TGGCAGCGTAGGGTAAGGT	0.29	0.37	0.78
ERCC1	CCTGCTTGTCAGGTGGAT GCTCCAGGCGAGGATCAAT	0.70	0.75	0.93
DPD	AAGCCAGGATACTCTCGATG GCTGTCACCTGGTCTCTG	0.84	1.12	0.75
SDHA	CCACTCGCTATTGCACACC CACTCCCCGTTCTCCATCA	gene referee		

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