Resistance to chemotherapy in non-small cell lung cancer with *Keap1* gene mutation

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Abstract We report the case of a 49-year-old Japanese man with advanced non-small cell lung carcinoma showing a *Keap1* gene mutation. Although he received platinum-based first- and second-line chemotherapies, he was judged to have progressive disease because of the appearance of new bone metastasis. Immunohistochemical analysis revealed the strong nuclear expression of NF-E2 related factor 2 (Nrf2) with higher expression of detoxification enzyme and drug efflux pump genes in the tumor cells. We detected a mutation associated with amino acid substitutions in Keap1 of the DGR/Kelch domain, the Nrf2 binding region. No *EGFR* gene or *KRAS* gene mutation was detected. These results suggest that a dysfunctional mutation of the *Keap1* gene caused constitutive activation of Nrf2. In this case, activation of Nrf2 might relate to chemoresistance via induction of its target genes, such as detoxification enzyme and drug efflux pump genes.

Keywords Non-small cell lung cancer · Nrf2 · Keap1 · Chemoresistance

Introduction

The transcription factor NF-E2 related factor 2 (Nrf2) is known as a pivotal factor for cell protection from oxidative

and electrophilic insults [1]. Nrf2 is highly expressed in non-small cell lung cancer (NSCLC) cells [2, 3]. Using NSCLC cell lines, we previously demonstrated that Nrf2 causes resistance to anticancer drugs by transactivating its target genes, including various detoxification enzymes, glutathione-related enzymes, and drug efflux pumps [4, 5]. Here, we show a case of chemoresistant NSCLC in which Nrf2 was highly activated because of dysfunctional mutation of the Kelch-like ECH-associated protein-1 (*Keap1*) gene.

Case report

A 49-year-old Japanese man was admitted to our hospital's neurosurgical department because of a recent episode of nausea, headache, and vertigo. He had a 20-year history of smoking. Family history was noncontributory. He had no abnormal findings on neurological examination on admission. Brain magnetic resonance imaging (MRI) showed a 30-mm gadolinium-enhanced lesion in the right cerebellum (Fig. 1a) which was radiologically diagnosed as a metastatic brain tumor. Levels of serum carcinoembryonic antigen (CEA), cytokeratin-19 fragments, squamous cell carcinoma antigen, and neuron-specific enolase were 135.2 ng/ml (reference range 0–5.0), 2.7 ng/ml (range 0–3.5), 0.7 ng/ml (range 0–1.5), and 7.3 ng/ml (range 0–12.0), respectively. The brain tumor was surgically resected to resolve his symptoms. The pathology finding of the surgical specimen was metastatic lung cancer (poorly differentiated NSCLC) (Fig. 2a). Computed tomography (CT) showed a 15-mm nodule as the primary lesion in S1 of the right lung (Fig. 1). He was diagnosed with NSCLC with the disease stage IV, cT1N2M1b, with right mediastinal lymph node metastasis and brain metastasis. As no

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Fig. 1 Clinical course. CT of the chest revealed a 15-mm nodule in S1 of right lung and right mediastinal lymph node metastasis (white arrows). Magnetic resonance imaging of the brain revealed a ring-enhancing lesion in the right cerebellum on admission. Bone

scintigram (bottom right) showed abnormal uptake of ^{99m}Tc -MDP in the right pelvis after treatment with second-line chemotherapy (black arrow). CDDP cisplatin, VNR vinorelbine, CBDCA carboplatin, PTX paclitaxel

mutations were detected in his epidermal growth factor receptor (*EGFR*) gene, he initially received cisplatin and vinorelbine as first-line chemotherapy. The first-line chemotherapy was not effective because of progression of mediastinal lymph node metastasis while the size of primary lesion did not change (Fig. 1). He received second-line chemotherapy with carboplatin and paclitaxel. However, second-line chemotherapy was also not effective, as new bone metastases appeared in his right pelvis (Fig. 1). Blood examination showed that the CEA level had increased to 431.6 ng/ml after second-line chemotherapy, up from 135.2 ng/ml before treatments. He was transferred to another hospital to receive palliative care.

Immunohistochemistry of the surgical specimen showed strong immunoreactivity to Nrf2 in the tumor cell nuclei (Fig. 2b). NAD(P)H-quinone oxidoreductase 1 (NQO1), a detoxification enzyme, and multidrug resistance-associated protein 1 (MRP1), a drug efflux pump—known as the Nrf2 target molecules—were also strongly expressed in tumor cells (Fig. 2c, d). Sequencing analysis of the tumor genome DNA revealed a single nucleotide mutation associated with

amino acid substitutions (GAT to TAT; D422Y) in the *Keap1* gene (Fig. 3; Table 1). However, no somatic mutations were detected in the *EGFR* or *KRAS* genes.

Discussion

Lung cancer is the leading cause of cancer-related death in developed countries [6]. The prognosis of lung cancer, especially in advanced NSCLC cases, remains unfavorable. A main cause of poor outcome in NSCLC treatment is innate resistance to anticancer drugs. Studies have demonstrated that NSCLC expresses products of several self-defense genes that protect against anticancer drugs, including phase II detoxifying enzyme genes, glutathione-related enzyme genes, and drug efflux protein genes [7]. Accumulating evidence also suggests that Nrf2 plays a critical role in resistance to anticancer drugs and tumor proliferation by transactivating its target genes via mediating antioxidant responsive element (ARE), a cis-acting enhancer element that is upstream of many antioxidant

Fig. 2 Hematoxylin and eosin staining (a) and immunostaining for Nrf2 (b), NQO1 (c), and MRP1 (d) in surgically resected specimen. Magnification ×100

Fig. 3 Sequence analysis of tumor genome DNA showed a single nucleotide mutation (G–T; arrow) associated with amino acid substitutions (D422Y) in *Keap1* gene

Table 1 Sequencing analysis of *Keap1*, *EGFR*, and *KRAS* genes

Gene	Nucleotide alteration	Amino acid alteration
<i>Keap1</i>	GAT to TAT (exon 3)	D422Y (DGR/Kelch)
<i>EGFR</i>	None	None
<i>KRAS</i>	None	None

enzymes, phase II detoxification enzymes, glutathione-related enzymes, and drug efflux pumps [4, 5]. In this case, correspondingly, strong expression of NQO1 and MRP1 was detected in cancer cells with higher expression of Nrf2. Although several mechanisms, such as loss of DNA mismatch repair, bypass of DNA adducts, and increased nucleotide excision repair and interstrand cross-link repair, have been reported to be involved in cisplatin resistance in cancer cells [8], activation of Nrf2 may thus have

contributed to resistance to platinum-based chemotherapy in this case. This is consistent with a previous finding that nuclear expression of Nrf2 in NSCLC plays a role in resistance to platinum-based treatment [9]. The role of Nrf2 in resistance to chemotherapeutic drugs has been demonstrated in other cancers, such as endometrial cancer [10] and epithelial ovarian cancer [11].

Activation of Nrf2 is mainly regulated by its inhibitory protein Keap1. Under resting conditions in normal cells, Nrf2 is retained in the cytoplasm by Keap1, and is maintained at a reduced level by Keap1-dependent ubiquitination and proteasomal degradation systems [12, 13]. Upon exposure to oxidative stress or xenobiotic stress, Keap1-dependent ubiquitin ligase activity is inhibited and Nrf2 can translocate to the nucleus, where it forms a heterodimer with small Maf proteins and binds to ARE. In the present case, we detected a single nucleotide mutation associated with amino acid substitutions (D422Y) in the *Keap1* gene. The mutation is located in the DGR/Kelch domain of Keap1. This domain is the Nrf2 binding region and cells possessing mutations in this region could not repress Nrf2 activity [3]. Beside Keap1, Nrf2 can be activated by phosphorylation signals directed by mitogen-activated protein kinase (MAPK) [14]. In cancer cells, these pathways are often activated via EGFR signaling pathways. A recent study also demonstrated that activating mutation of *kras* activates Nrf2 via MAPK pathways [15]. We therefore examined whether activation of Nrf2 is due to activating mutations of the *EGFR* or *KRAS* genes. However, there was no mutation in either gene. Thus, it is likely that activation of Nrf2 in this case is mainly due to the dysfunctional mutation in the *Keap1* gene.

Ohta et al. [2] demonstrated that *Keap1* somatic mutations associated with amino acid substitutions were detected in 7.7% (5 of 65) of Japanese lung cancer patients; all of the mutations were detected in NSCLC, and 4 of 5 were in the DGR/Kelch domain. Takahashi et al. demonstrated that *Keap1* mutations with amino acid changes were detected in 5.1% (4 of 79) of Japanese NSCLC patients and all of them were located in the DGR/Kelch domain [16]. On the other hand, *Keap1* gene mutations were detected in 19% (10 of 54) of Caucasian lung cancer patients [3]. Although the *Keap1* gene mutation in lung cancer was less common in Japanese patients than in Caucasian patients, almost all such mutations found in Japanese patients may be dysfunctional mutations because they were located in the DGR/Kelch domain. *Keap1* gene mutations are suggested to be associated with worse prognoses and lower postoperative disease-free survival rates in resected stage I–II NSCLC [16]. Thus, Nrf2/Keap1 might be a useful therapeutic target for patients with Keap1-mutated NSCLC. Moreover, Nrf2 is thought to be a critical molecular target to enhance the efficacy of chemotherapy

because a recent study demonstrated that brusatol, an inhibitor of the Nrf2 pathway, sensitizes a broad spectrum of cancer cells to cisplatin and other chemotherapeutic drugs [17].

Conflict of interest None.

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