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Case report

Does V600E *BRAF* mutation predict vinorelbine efficacy? A proof-of-concept from a lung micropapillary adenocarcinoma metastatic to the breast

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Key words

Lung • Adenocarcinoma • Breast • Metastasis • Micropapillary • BRAF • V600E • Vinorelbine

Summary

BRAF mutations occur in about 3% of all lung adenocarcinomas and V600E missense mutation characterizes about half of *BRAF*-mutated lung adenocarcinomas and is significantly associated with micropapillary pattern and shorter disease-free and overall survival rates. In this report, we report a challenging case of a patient with a metastatic micropapillary adenocarcinoma of the lung harbouring V600E *BRAF* mutation who experienced a surprising protracted clinical response to metronomic vinorelbine. The possible association between the V600E *BRAF* mutation pathway and the effective use of vinca alkaloid is discussed.

Introduction

BRAF mutations occur in about 3% of all lung adenocarcinomas [1]. V600E missense mutation characterizes 55-60% of *BRAF*-mutated lung adenocarcinomas and is significantly associated with the female gender, micropapillary pattern at histology and shorter disease-free and overall survival rates [1]. According to the new WHO classification [2], invasive adenocarcinoma with micropapillary pattern tends to spread through airspaces with a greater capacity for local and lymphangitic infiltration. Together with solid type, micropapillary pattern is independently associated with patients survival in surgically-resected adenocarcinomas and seems to better respond to chemotherapy in adjuvant setting [3 4].

Most interestingly, a recent work by Vecchione et al. [5] demonstrated that "in vitro" and "in vivo" V600E *BRAF* mutant colon cancer cells demonstrate a significant sensitivity to vinorelbine, possibly through inhibition of a defective activity of RANBP2, a small GTP-binding protein belonging to the RAS superfamily regulating the nucleo-cytoplasmic transport and stabilizing the kinetochore function during mitotic activity.

Vinorelbine is a semisynthetic vinca alkaloid that binds to tubulin, thus inhibiting mitotic microtubule polymerization and demonstrating a radioenhancer cell cycle-dependent activity on tumor cells, exerting its widest efficacy in cell killing during the G2/M phase of the cell cycle [5 6]. Oral administration of vinorelbine has shown a good clinical safety profile, but no biomarker has been identified in predicting vinorelbine efficacy [6].

Nevertheless, previous works have observed a major clinical response of some chemotherapeutic agents in small subsets of molecular-driven adenocarcinomas, namely pemetrexed in ALK and ROS1 rearranged tumors possibly related to high content of thymidylate synthase, one of the subcellular target of pemetrexed [7].

In this report, we describe a metastatic micropapillary adenocarcinoma of the lung simulating an inflammatory cancer of the breast, harbouring V600E *BRAF* mutation and lacking RANBP2 expression. The patient experienced a surprising protracted clinical response to metronomic vinorelbine. The possible association between the V600E *BRAF* mutation pathway and the effective use of vinca alkaloid is discussed.

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Case report

A 80 year-old woman, current smoker, presented with a diffuse swelling of the left breast associated with skin reddening and pain from several weeks. She had a previous history of primary lung adenocarcinoma resected 4 years before (left lower lobe, stage IIA; pT1N1). She also suffered from autoimmune thyroiditis and vascular hypertension.

Clinical examination of the breast showed erythematous, thickened and edematous skin suggesting carcinomatous mastitis. No palpable nodules were noted. No axillary lymphadenopathy was observed and the right breast looked entirely normal.

Routine laboratory tests were unremarkable, while CEA was significantly increased (1366 ug/L). CA15.3 and CA19.9 serum tumor markers showed normal levels.

Ultrasound echography of the left breast revealed diffuse tissue edema with hypertrophy of the mammary parenchyma. Mammography showed diffuse increase of the breast density associated with scattered microcalcifications and marked thickening of the skin (Fig. 1A). No alterations were noted in the right breast. A core-biopsy using a 14G needle was performed. Histologic examination showed fragments of adipose tissue with lymphatic vessels engulfed by an adenocarcinoma with micropapillary pattern associated with psammomatous microcalcifications (Fig. 1B).

At immunohistochemistry, the neoplastic cells expressed TTF-1 (clone 8G7G3/1, Ventana Medical Biosystem, Tucson, AZ, USA) and napsin A (clone MRQ60, Ventana), whereas estrogen (clone SP1, Ventana) and progesterone (clone 1E2, Ventana) receptors were negative (Fig. 1C).

The comparison of morphology and immunoprofile between the primary lung adenocarcinoma and the breast tumor revealed an identical phenotype (Fig. 1D-E). Thus, a diagnosis of breast metastasis from lung adenocarcinoma with micropapillary pattern was made.

At molecular analysis, neither *EGFR* mutations nor *ALK* rearrangement were detected. However, the presence of the micropapillary pattern prompted us to investigate *BRAF* gene alterations and the missense V600E mutation was identified either in primary lung cancer and breast metastasis (Fig. 2A).

Tumor cells also expressed BRAF V600E mutation-specific antibody (clone VE1, Ventana) at immunohistochemistry (Fig. 2B), while immunostaining with RANPB2 (mAb58385, Abcam, Cambridge, UK) was negative (Fig. 2C).

A 18-FDG PET scan revealed the presence of hypermetabolic uptake in the left breast, mediastinal lymph nodes, pleura, ribs and humerus.

Given the poor conditions of the patient (ECOG PS:2) and the impossibility to use specific BRAF inhibitors in routine practice, chemotherapy with metronomic oral vinorelbine at the dose of 50 mg (one capsule of 20 mg plus one of 30 mg) three times weekly on Monday, Wednesday and Friday was started continuously until disease progression, patient refusal or excessive toxicity. At the same time, radiotherapy was performed to relieve bone pain. The patient promptly experienced general improvement of both mammary (**Fig. 2D**) and bone symptoms and she is still alive with disease in good conditions (ECOG PS:1) at 9 month's follow up from chemotherapy starting.

Fig. 1. Breast mammography showed a diffuse enlargement of mammary parenchyma with microcalcifications (A) due to lymphangitic infiltration of a metastatic adenocarcinoma with micropapillary pattern and numerous psammomatous calcifications (B, haematoxylin-eosin stain) expressing TTF-1 (C). Comparison of morphology (D, haematoxylin-eosin) and immunoprofile (E, TTF-1 immunostain) of the previously resected lung adenocarcinoma showed an identical phenotype (micropapillary pattern with psammomas and TTF-1 immunoreactivity).

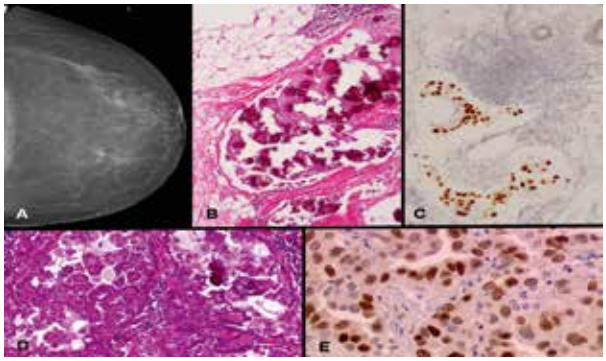
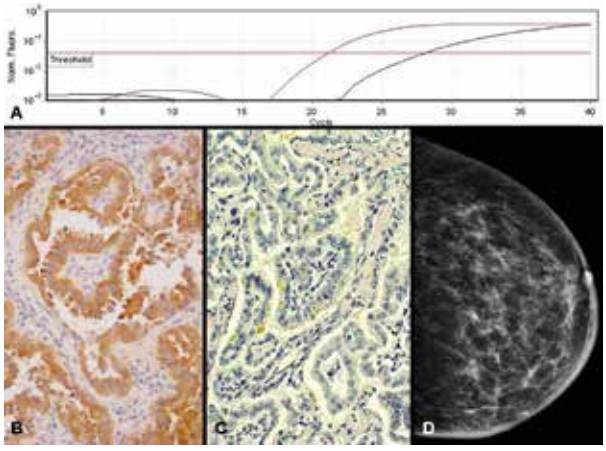


Fig. 2. RT-PCR molecular analysis showed V600E BRAF mutation (A) further confirmed by espressione of V600E BRAF mutation-specific antibody (B, VE1 immunostain). Tumor cells were negative for RANPB2 (C). Breast mammography after 7 months from chemotherapy starting showed a decrease of mammary volume with fibrotic density (D).



Discussion

There are two interesting clues characterizing the case here described. Firstly, the clinical presentation of a metastatic lung adenocarcinoma with micropapillary pattern involving the breast and strikingly mimicking an inflam-

matory carcinoma. Our case is very similar to that described by Jeong et al. [8] reporting a 47 year-old woman with a pulmonary metastatic micropapillary adenocarcinoma to the breast after 3 years from the lung resection. Since micropapillary growth pattern is observed even in primary breast carcinoma, the differential diagnosis may be challenging, requiring the knowledge of the patient's clinical history, results of imaging and laboratory studies and comparison of morphology, immunoprofile and molecular features between primary and metastatic tumors. Although lung cancer metastatic to the breast generally pursues a dismal outcome, a correct diagnosis is mandatory to prevent unnecessary breast surgery and properly leading to investigate predictive biomarkers, then permitting alternative effective therapies using targeted molecules with a low-toxicity profile In their case, Jeong et al. [8] evidenced the presence of *EGFR* mutation in exon 19 (L747-E749del) and the patient experienced a long-lasting survival (disease-free after 23 months from the diagnosis of breast metastasis) under gefitinib.

Similarly to *EGFR* mutations, Marchetti et al. [1] evidenced that micropapillary component is a characteristic growth pattern (80%) also in pulmonary adenocarcinoma harbouring V600E *BRAF* mutation. Indeed, the second point of discussion emerging from the case herein concerns the good clinical response to vinorelbine correlated to the presence of V600E *BRAF* mutation.

Although no biomarkers have been identified in predicting vinorelbine efficacy in lung cancer, an interesting study by Vecchione et al. [5] elegantly demonstrated in preclinical models that V600E *BRAF*-mutated colon cancer cells were significantly inhibited by vinorelbine through depletion of RANBP2, an essential tumor suppressor gene responsible of the interaction of kinetochores with the microtubule bundles that extend from the centrosomes to the kinetochores during mitosis and maintaining chromosome stability. Depletion of RANBP2 leads to abnormal mitotic progression, and abnormal chromosome segregation and seems to characterize V600E *BRAF* mutated colon cancer, then representing a vulnerability point for microtubule disrupting agents, as vinca alkaloid.

Although only at immunohistochemistry level, according to these latter observations [5], we found that tumor cells of the primary lung and metastatic adenocarcinomas were negative for RANBP2.

In lung cancer, RANBP2 expression has been poorly investigated, but the great majority of NSCLC cell lines show up-regulation of this protein [9].

In summary, we reported a metastatic micropapillary pulmonary adenocarcinoma clinically mimicking an inflammatory breast cancer after 4 years from surgery. A significant clinical response to metronomic vinorelbine was observed and the tumor cells evidenced V600E *BRAF* mutation coupled to defective RANBP2 protein. Whether these molecular alterations could predict vinorelbine efficacy in lung cancer, as recently demonstrated in preclinical models of colon cancer, clearly require further investigations.

Waiting for the official approval of effective V600E *BRAF* mutation-specific inhibitors [10], patients with lung adenocarcinoma harboring V600E *BRAF* mutation could receive a clinical benefit from a vinorelbine-based chemotherapy regimen.

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