Tyrosine kinase activity profiling of metastatic malignant melanoma: Identification of possible therapeutic targets and markers predicting response to therapy

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Abstract

Metastatic melanoma is an aggressive form of cancer that responds poorly to conventional chemotherapy. The aim of this study was to obtain information about the tyrosine kinase activity in metastatic malignant melanoma in the presence and absence of two different kinase inhibitors. Tyrosine kinase activity was measured in 26 human metastatic stage IV melanoma samples and 4 normal skin samples using Tyrosine Kinase PamChip® arrays. The overall protein tyrosine kinase (PTK) activity was higher in melanoma compared to normal skin. Further, we attempted to evaluate the tyrosine kinase activity in response to the B-Raf inhibitor PLX4032, and the multi-targeted kinase inhibitor sunitinib, in order to receive important tumor-specific information on affected targets and pathways. Differential kinase activity was observed between BRAFwt and BRAFV600E in response to PLX4032. Based on the inhibition profiles, 41 peptide substrates were identified as significantly different between the two groups, with many of the peptides encoding for proteins involved in the MAPK pathway. Stronger inhibition was observed in tumors with BRAFV600E compared to BRAFwt. Treatment with sunitinib caused the expected pattern of kinase inhibition.

Introduction

Metastatic malignant melanoma is a life-threatening cancer type lacking optimal systemic treatment. Established standard chemotherapy with dacarbazine (DTIC) has shown poor response rates (10-20%) in patients with advanced melanoma, and more targeted therapies are urgently needed. Kinases are important key regulators of the cell, and the use of kinase inhibitors as cancer treatment has increased dramatically. In 2011, vemurafenib (PLX4032) was approved by the FDA for the treatment of advanced melanoma. Vemurafenib is a serine/threonine kinase inhibitor targeting B-Raf with V600E mutation. This type of mutation occurs in ~50% of all melanomas, and vemurafenib has shown positive results in patients harboring BRAFV600E from late stage melanoma (1). However, the occurrence of de novo and acquired resistance towards vemurafenib in parallel with the V600E mutation needs further investigation on the molecular mechanisms involved. We studied the kinase activity profiles in metastatic malignant melanoma in the presence and absence of either PLX4032 or sunitinib. Our aim was to identify PTKs that are deregulated in metastatic malignant melanoma and possibly identify novel targets and pathways involved in resistance to the tested TKIs.

Materials and Methods

Tyrosine Kinase PamChip® array technology

Tyrosine kinase activity profiling was performed on fresh frozen tumor tissue from 26 stage IV melanoma patients prior to DTIC treatment. All specimens have previously been described and screened for mutations in BRAF, NRAS, CDKN2A and TP53 (3). In addition, 4 normal skin tissue samples were collected from the skin of individuals not affected by melanoma. The tissue was digested in cupides and lysed in lysis buffer supplemented with phosphatase and protease inhibitors for inhibition profiles, 41 peptides could differentiate between normal samples, and were identified as significantly different between the two groups.

Results

Tyrosine kinase activity in metastatic malignant melanoma

Fig.1 Tyrosine Kinase PamChip Array for PamStation412 consists of 144 peptide substrates with known phosphorylation sites, representing 160 different proteins. The technology allows automated, fast and easy identification of substrates for tyrosine kinases, and provides for the multi-parametric detailed analysis of the kinetic mechanism of kinases (2). Samples and data analysis

Tyrosine kinase activity profiling was performed on fresh frozen tumor tissue from 26 stage IV melanoma patients prior to DTIC treatment. All specimens have previously been described and screened for mutations in BRAF, NRAS, CDKN2A and TP53 (3). In addition, 4 normal skin tissue samples were collected from the skin of individuals not affected by melanoma. The tissue was digested in cupides and lysed in lysis buffer supplemented with phosphatase and protease inhibitors for inhibition profiles, 41 peptides could differentiate between normal samples, and were identified as significantly different between the two groups.

Discussion

Our results show that the PTK activity is generally higher in metastatic melanoma compared to normal skin. In the PLX4032 inhibition profile, 41 peptides could differentiate between BRAFwt and BRAFV600E samples. Most of the peptides encoded for proteins involved in the MAPK pathway, previously identified to be important for the resistance and response to PLX4032 (4,5). Upregulated tumor expression of EGFR has been suggested to play a role in resistance towards PLX4032 in colon cancer patients with BRAFV600E mutations (4). In melanoma, acquired resistance to PLX4032 is thought to develop through upregulated PDGFRα (5). Taken together, the results suggest that PTKs involved in the MAPK pathway may serve as important targets for treatment of metastatic malignant melanoma. The finding of human melanoma samples with BRAFwt behaving like BRAFV600E in response to treatment with PLX4032 (Fig.4) may indicate that some patients could benefit from treatment with PLX4032, despite lacking the V600E mutation. This demands further clarification in the future.

References