Biochemical and Biophysical Research Communications 398 (2010) 585–587

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

IDH1^{R132} mutation identified in one human melanoma metastasis, but not correlated with metastases to the brain

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ARTICLE INFO

Article history:
Received 10 June 2010
Available online 13 July 2010

Keywords:
Isocitrate dehydrogenase 1
Isocitrate dehydrogenase 2
Melanoma
Brain tumors metastases

ABSTRACT

Isocitrate dehydrogenase 1 (*IDH1*) and *isocitrate dehydrogenase 2* (*IDH2*) are enzymes which convert isocitrate to α-ketoglutarate while reducing nicotinamide adenine dinucleotide phosphate (NADP + to NADPH). *IDH1/2* were recently identified as mutated in a large percentage of progressive gliomas. These mutations occur at *IDH1*^{R132} or the homologous *IDH2*^{R172}. Melanomas share some genetic features with *IDH1/2*-mutated gliomas, such as frequent *TP53* mutation. We sought to test whether melanoma is associated with *IDH1/2* mutations. Seventy-eight human melanoma samples were analyzed for *IDH1*^{R132} and *IDH2*^{R172} mutation status. A somatic, heterozygous *IDH1* c.C394T (p.R132C) mutation was identified in one human melanoma metastasis to the lung. Having identified this mutation in one metastasis, we sought to test the hypothesis that certain selective pressures in the brain environment may specifically favor the cell growth or survival of tumor cells with mutations in *IDH1/2*, regardless of primary tumor site. To address this, we analyzed *IDH1*^{R132} and *IDH2*^{R172} mutation status 53 metastatic brain tumors, including nine melanoma metastases. Results revealed no mutations in any samples. This lack of mutations would suggest that mutations in *IDH1*^{R132} or *IDH2*^{R172} may be necessary for the formation of tumors in a cell-lineage dependent manner, with a particularly strong selective pressure for mutations in progressive gliomas; this also suggests the lack of a particular selective pressure for growth in brain tissue in general. Studies on the cell-lineages of tumors with *IDH1/2* mutations may help clarify the role of these mutations in the development of brain tumors.

1. Introduction

Isocitrate dehydrogenase 1 (*IDH1*) and *isocitrate dehydrogenase 2* (*IDH2*), which convert isocitrate to α-ketoglutarate while reducing nicotinamide adenine dinucleotide phosphate (NADP+ to NADPH), were identified as mutated in a large percentage of progressive gliomas and acute myeloid leukemias [1–5]. These mutations occur at the R132 residue in *IDH1* or the homologous R172 residue in *IDH2*. Melanomas share some genetic features with *IDH1/2*-mutated gliomas, such as frequent *TP53* mutation [6]. Previously, no *IDH1*^{R132} mutations were found in a group of 23 melanomas [7], and to our knowledge, melanoma has not been analyzed for *IDH2*^{R172} mutations. We sought to test whether melanoma is associated with *IDH1/2* mutations by analyzing a larger group of samples. In addition, given the finding of high *IDH1/2* mutation frequency (80%) in progressive gliomas, we sought to test the hypothesis that certain selective pressures in the brain environment may favor cell growth/survival of tumor cells with mutations in *IDH1/2*. Therefore, to address whether mutation of *IDH1/2* might be required for the development of brain metastases from non-primary central nervous system (CNS) tumors, we sequenced *IDH1*^{R132} and *IDH2*^{R172} in 53 metastatic brain tumors, including melanomas.

2. Materials and methods

IDH1/2 gene mutation status was first analyzed in a panel of cell lines derived from human non-CNS metastatic melanoma tumor resections, paired with pheresis-collected peripheral blood

Abbreviations: CNS, central nervous system; *IDH1*, *isocitrate dehydrogenase 1*; *IDH2*, *isocitrate dehydrogenase 2*.

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0006-291X/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2010.06.125

mononuclear cells from 78 patients enrolled in Institutional Review Board-approved clinical trials at the Surgery Branch of the National Cancer Institute at the United States National Institutes of Health. Pathology-confirmed melanoma cell lines were derived from mechanically or enzymatically dispersed tumor cells, which were then cultured for 5–15 passages. *IDH1/2* gene mutation status was subsequently analyzed in a panel of 53 tumor resections from metastases to the brain. Genomic DNA was isolated from frozen tumor tissue samples obtained from the Tissue Bank at the Preston Robert Tisch Brain Tumor Center at Duke University. In both cases, genomic DNA was isolated using the DNeasy Blood and Tissue kit (Qiagen, Valencia, CA, USA). PCR and sequencing primers were designed using Primer 3 (http://www-genome.wi.mit.edu/cgibin/primer/primer3_www.cgi) and synthesized by Invitrogen (Carlsbad, CA, USA) and Integrated DNA Technologies (Coralville, IA, USA). PCR primers were designed to amplify the selected *IDH1* and *IDH2* exons. PCR products were 300–500 bp in length. PCR primers were designed to amplify the region surrounding the commonly mutated codons in both *IDH1* and *IDH2*. In one melanoma sample, all coding exons of *TP53*, *CDKN2A,* and *CDKN2B*, as well as hotspot exons of *BRAF* and *NRas*, were sequenced using these methods.

3. Results and discussion

Of 78 human melanoma samples analyzed for $IDH1^{R132}$ and $IDH2^{R172}$ mutation status, a somatic, heterozygous *IDH1* c.C394T (p.R132C) mutation was identified in tissue derived from a melanoma lung metastasis from a 53 year old female (Fig. 1). No $IDH2^{R172}$ mutations were detected in any of these samples. In the *IDH1*-mutated sample, we sequenced regions of *BRAF*, *NRas*, *TP53*, and *CDKN2A/CDKN2B* to identify any changes in genes that are commonly altered in melanoma. We found a *BRAF* c.T1799A (p.V600E) mutation, while *NRas*, *TP53*, and *CDKN2A/CDKN2B* were unaltered in this sample.

IDH1/2 mutations are frequent in progressive gliomas, but are very rare in other cancers besides acute myelogenous leukemia. To date, mutations in *IDH1* have been identified only in one B-cell acute lymphoblastic leukemia [8], two prostate cancers [8], and one colorectal cancer [9]. Noting the presence of mutations in this small fraction of other cancers, we postulated two hypotheses. In the first hypothesis, mutations in *IDH1/2* may be cell-lineage dependent, and thus only occur in the cell-of-origin for a specific subset of tumors, including progressive gliomas. In the second hypothesis, a tissue-dependent selective pressure for growth in brain tissue may select for frequent mutations in *IDH1/2*. If the second hypothesis is correct, we should be able to identify the presence of relatively frequent mutations in *IDH1/2* in non-primary CNS metastases to the brain. Therefore, we analyzed $IDH1^{R132}$ and $IDH2^{R172}$ mutation status in a set of nine melanoma metastases to the brain and an additional panel of 44 metastases of other tumor types to the brain, including lung, breast, renal, uterine, ovarian, esophageal, and urothelial cancer metastases. Results revealed no mutations regardless of tumor type, consistent with a previous small-scale study looking at colon cancer metastases to the brain [10].

Here, we present the first identification of a mutation in *IDH1* or *IDH2* in melanoma. These results have important clinical implications regarding the role of mutations in *IDH1/2* in the development of tumors. Mutations in *IDH1* have now been identified in gliomas, leukemias, prostate cancer, colorectal cancers, and melanomas, suggesting the possibility of a commonly altered pathway that may prove advantageous to the formation of tumors in all these cell types. The lack of mutations in a panel of non-primary CNS metastases to the brain would suggest that mutations in $IDH1^{R132}$ or $IDH2^{R712}$ are not necessarily required specifically for growth in brain tissue. The low frequency of mutations in $IDH1^{R132}$ and $IDH2^{R172}$ in primary glioblastomas, which grow extremely aggressively in brain tissue, further supports this. This leads to the conclusion that mutations in these genes may be necessary for formation of tumors in a cell-lineage dependent manner, with a particularly strong selective pressure for mutations in progressive gliomas and acute myelogenous leukemias. Studies focusing on the cell-lineages from which tumors with *IDH1* or *IDH2* mutations develop may help to elucidate the role of these mutations in cancer pathogenesis.

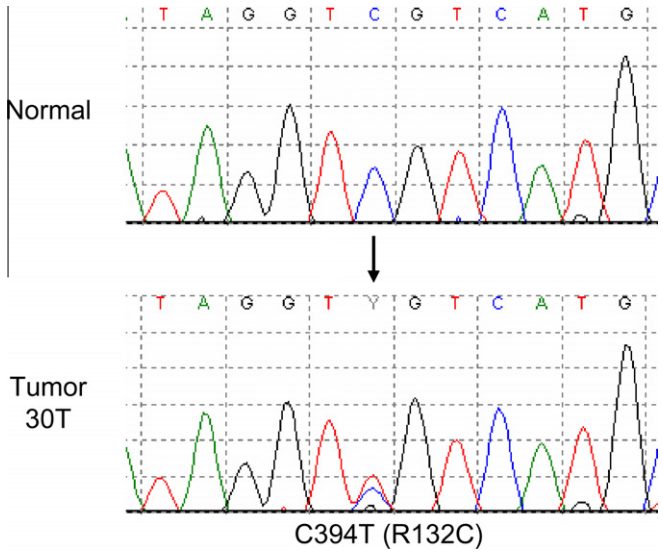

Fig. 1. Sequencing of melanoma metastases identified IDH1 R132C mutation in a melanoma cell line derived from a melanoma lung metastasis.

Acknowledgments

We thank Kristin E. Yates for technical assistance. This work is supported by the Intramural Research Programs of the National Human Genome Research Institute and the National Cancer Institute, National Institutes of Health, USA, and by NIH grant R01CA118822.

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