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ORIGINAL ARTICLE

Immunohistochemically detected IDH1^{R132H} mutation is rare and mostly heterogeneous in prostate cancer

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Abstract

Background IDH1 mutations are oncogenic through induction of DNA damage and genome instability. They are of therapeutic interest because they confer increased sensitivity to radiation and cytotoxic therapy and hold potential for vaccination therapy.

Methods In this study, we analyzed more than 17,000 primary prostate cancer tissues with a mutation-specific antibody for the IDH1^{R132H} mutation.

Results IDH1 mutation-specific staining was found in 42 of 15,531 (0.3%) interpretable cancers. IDH1 mutation was associated with higher preoperative PSA and Gleason grade ($p < 0.05$, each) but was unrelated to PSA recurrence. A comparison with other molecular tumor features available from earlier studies revealed that *TMPRSS2-ERG* fusion as well as deletion of *PTEN*, 5q21, 6q15, and 3p13 was less frequent in IDH1-mutated than in non-mutated cancer. Increased lethality of genetically instable, "aberration-rich" cancer cells in the presence of IDH1 mutations could possibly explain this observation. Heterogeneity analysis revealed a homogeneous mutation in only 1 of 16 IDH1-mutated cancers. This high degree of heterogeneity may profoundly limit therapeutic targeting of IDH1 mutations in prostate cancer.

Conclusions The data show that 0.3% of prostate cancers have an IDH1^{R132H} mutation and that these are mostly heterogeneous. Once specific anti-IDH1 therapy becomes reality, only a very small group of prostate cancer patients may benefit from such a treatment.

Keywords IDH1 · ERG · Deletion · Prostate cancer · TMA

Introduction

Isocitrate dehydrogenase 1 (IDH1) is an enzyme involved in the citrate cycle. IDH1 catalyzes the conversion of isocitrate to α-ketoglutarate (α-KG) with release of nicotinamide adenine dinucleotide phosphate (NADPH), a key molecule for energy production and an essential reducing factor required for cellular defense mechanisms against oxidative damage [1]. IDH1 has gained considerable interest in 2008, when a specific hotspot mutation Arg132His (IDH1^{R132H}) was discovered in human glioblastoma [1]. Subsequent studies revealed that this IDH1 mutation occurs most frequently in particular subtypes of brain cancer, including > 80% of low-grade glioma and secondary glioblastoma [2]. IDH1 mutations are believed to represent early and potentially cancer-initiating events in these subtypes [3], and have been linked

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to other specific genetic alterations including deletion of chromosome 1p/19p and p53 mutation [4]. The IDH1^{R132H} mutation results in a neo-enzymatic function leading to the synthesis of D-2-hydroxyglutarate (2-HG) instead of α-KG, a process that consumes NADPH instead of synthesizing it [5]. High levels of 2-HG are believed to exert oncogenic functions in at least two ways, i.e., modification of epigenetic control through inhibition of α-KG-dependent histone- and DNA-demethylases [6, 7], as well as induction of DNA damage and genome instability as a consequence of lowered cellular NADPH levels [8].

The different function of mutant IDH1 may hold promise for novel therapeutic approaches in several ways: The association of IDH1 mutations with DNA hypermethylation raises the possibility that hypomethylating agents may be effective against IDH-mutated cancers [8]. Moreover, the hotspot nature of mutant IDH1 makes it a highly promising candidate for novel immunotherapy and vaccination strategies. In addition, cell line models of glioblastoma suggest that IDH1-mutated cells with low NADPH levels are sensitive to irradiation and chemotherapy [9], which might explain the prolonged survival of patients with IDH1-mutated glioblastoma [10]. Accordingly, the IDH1 mutation status is now routinely assessed in brain tumors.

IDH1 mutations are not limited to brain cancer, but do also occur in 50–70% of malignant chondrosarcomas [11], 5–15% of acute myeloid leukemias [12] and at least occasionally in melanoma [13] thyroid cancer [14], breast [15] and prostate cancer [16]. In a recent study performing next generation sequencing on 453 prostate cancers, IDH1 mutations were found in 1% of tumors. Because of the absence of other key molecular features in IDH1-mutated cancers, IDH1 mutation was suggested to define a molecular subgroup [17]. Based on the observation in brain cancer, it is tempting to speculate that IDH1 mutations could identify prostate cancers with increased response rate to radiotherapy. To learn more about the prevalence of IDH1 mutations, their role in tumor initiation and progression, and possible association to molecularly defined subsets of the disease, we employed an IDH1 mutation-specific commercial antibody to stain our prostate cancer tissue microarray containing more than 17,000 samples.

Materials and methods

Patients

Radical prostatectomy specimens were available from 17,747 consecutive patients, operated at the Department of Urology and the Martini Clinic of the University Medical Center Hamburg-Eppendorf between 1992 and 2014. The specimens were analyzed as described before [18]. Histopathological data were retrieved from the patients' record, including tumor stage, Gleason grade, nodal and resection margin status. In addition to the classical Gleason categories, "quantitative" Gleason grading was performed as detailed in [19]. Follow-up was available for 12,579 patients with a median follow-up of 48 months (range 1–276 months). Postoperative prostate-specific antigen (PSA) level of 0.2 ng/ml and higher was defined as PSA recurrence. The tissue microarray (TMA) had a spot size of 0.6 mm and contained various internal controls (e.g., normal prostate). The attached molecular database contained results on ERG expression, *ERG* break apart fluorescence in situ hybridization (FISH) analysis, deletion status of 5q21 (*CHD1*), 6q15 (*MAP3K7*), 10q23 (*PTEN*) and 3p13 (*FOXP1*).

Immunohistochemistry

Freshly cut TMA sections were stained on 1 day and in one experiment. Slides were deparaffinized and exposed to heat-induced antigen retrieval for 5 min at 121 °C in Tris–EDTA-citrate buffer pH 7.8. The mouse monoclonal antibody DIA H09 (Dianova, Hamburg, Germany; dilution 1:20) specific for IDH1^{R132H} was applied at 37 °C for 60 min. Bound antibody was visualized with the EnVision Kit (Dako, Glostrup, Denmark). The IDH1^{R132H} specific antibody typically stained the cytoplasm in all (100%) tumor cells of a positive tissue spot (Fig. 1).

Statistics

JMP 12.0 software (SAS Institute Inc., NC, USA) was used. Contingency tables were calculated to study association between IDH1^{R132H} expression and clinico-pathological variables, and p values were obtained with the Chi square (likelihood) test. Kaplan–Meier curves were generated using biochemical (PSA) recurrence as the clinical endpoint and the log-rank test was used for p values.

Results

IDH1^{R132H} staining

A total of 15,531 (87%) of tumor samples were interpretable in our TMA analysis (Table 1). Reason for 2221 (13%) non-informative cases was lack of tissue sample or absence of unequivocal cancer tissue in the TMA spot. Normal prostate glands or stromal tissue did not show any IDH1^{R132H} staining under the selected experimental conditions. Also the vast majority of cancers lacked positive staining. Only 42/15,531 (0.3%) tumors showed IDH1^{R132H} staining. Staining was clear cut, involved 100% of cancer

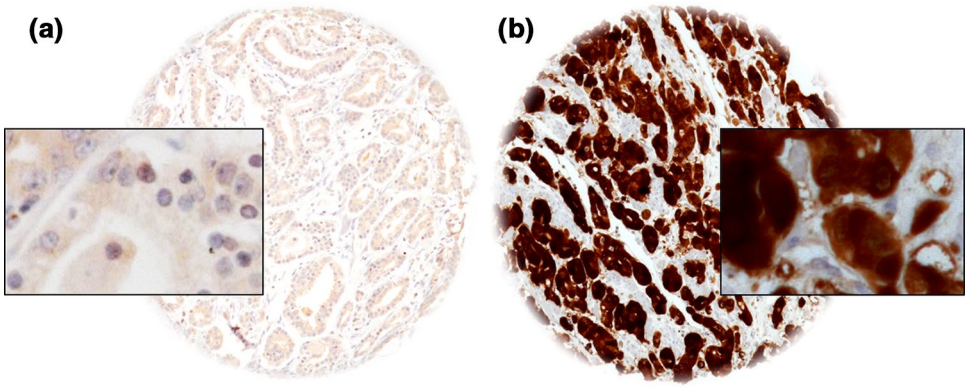

Fig. 1 Representative pictures of **a** negative and **b** positive IDH1^{R132H} staining in prostate cancer at ×100 and ×400 (inset) magnification with an original spot size of 0.6 mm

Table 1 Association between IDH1^{R132H} staining and prostate cancer phenotype

Parameter	*N* evaluable	IDH1^{R132H} positive (%)	*p* value
All cancers	15,531[a]	42 (0.3)	
Tumor stage			
pT2	9947	27 (0.3)	0.7741
pT3a	3445	8 (0.2)	
pT3b–pT4	2077	7 (0.3)	
Gleason grade			
≤ 3 + 3	2955	1 (0.03)	0.0128
3 + 4	8212	24 (0.3)	
3 + 4 Tert. 5	732	2 (0.3)	
4 + 3	1514	9 (0.6)	
4 + 3 Tert. 5	1078	3 (0.3)	
≥ 4 + 4	898	3 (0.3)	
Lymph node metastasis			
N0	9366	27 (0.3)	0.4607
N +	1146	2 (0.2)	
Preoperative PSA level (ng/ml)			
< 4	1909	1 (0.1)	0.0232
4–10	9179	22 (0.2)	
10–20	3244	13 (0.4)	
> 20	1105	6 (0.5)	
Surgical margin			
Negative	12,414	31 (0.2)	0.3141
Positive	3062	11 (0.4)	

[a]Total *N* can be smaller in subcategories

cells and was of moderate to strong intensity (Fig. 1). In order to study whether staining was homogeneous in IDH1^{R132H} positive cancer, we selected 16 IDH1^{R132H} positive cancers and analyzed either conventional large section (5 patients) or 0.6 mm tissue spots (11 patients) from all tumor containing tissue blocks. 16/16 tumors showed heterogeneous staining with the presence of both IDH1^{R132H} positive and negative tumor areas.

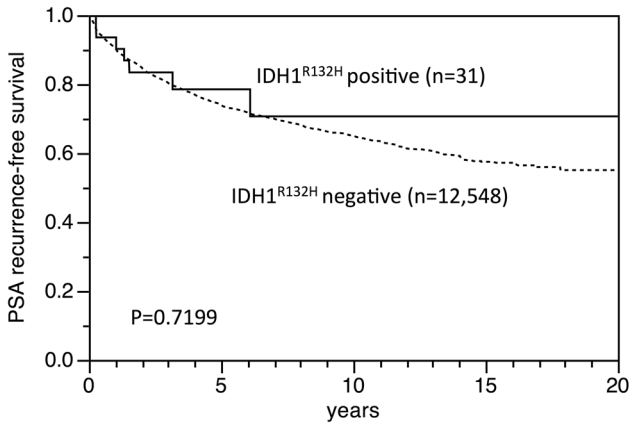

Fig. 2 Prostate-specific antigen (PSA) recurrence-free survival in IDH1^{R132H} positive and negative cancer

Association with tumor phenotype and patient outcome

The relationship between IDH1^{R132H} staining and prostate cancer phenotype is shown in Table 1. Positive IDH1^{R132H} staining was statistically linked to high preoperative PSA levels ($p = 0.0232$) and—due to its absence in Gleason 3 + 3 = 6 cancers—also to high Gleason grade ($p = 0.0128$). Follow-up data were available for 12,579 patients with interpretable IDH1^{R132H} staining on the TMA. IDH1^{R132H} staining was unrelated to PSA recurrence (Fig. 2).

Association with *TMPRSS2:ERG* fusion status and chromosomal deletions

Data on *TMPRSS2:ERG* fusion status obtained by FISH were available from 6333 and by immunohistochemistry from 9464 tumors with evaluable IDH1^{R132H} staining. Data on both ERG FISH and IHC were available from 6090 cancers, and an identical result (ERG IHC positive and break by FISH or ERG IHC negative and missing break by FISH) was found in 5804 of 6090 (95.3%) cancers.

IDH1^{R132H} positive cancers were less often ERG positive than IDH1^{R132H} negative cancers. This was valid for both FISH and IHC evaluation of the ERG status ($p < 0,05$ each; Fig. 3). The comparison of IDH1^{R132H} staining with *PTEN*, 5q21, 6q15, and 3p13 deletions revealed that every deletion was less common in IDH1^{R132H} positive cancers. However, these differences did not reach statistical significance (Fig. 4).

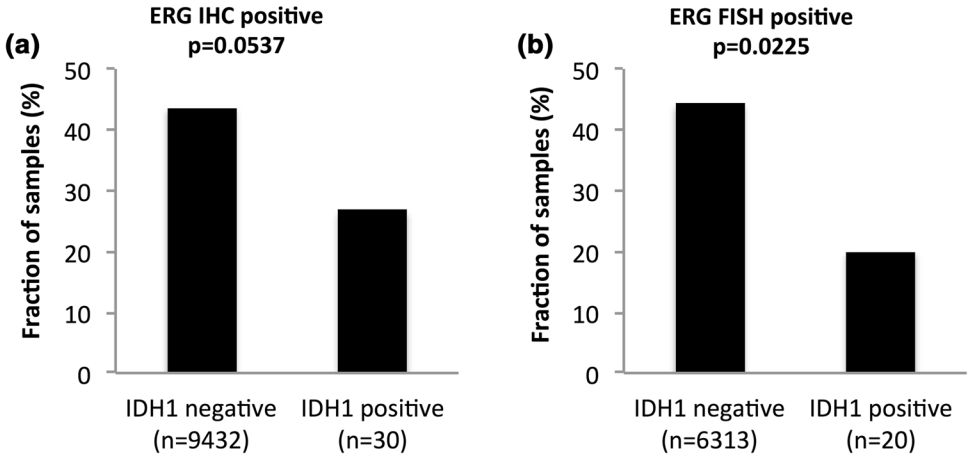

Fig. 3 Association between IDH1R132H staining and ERG status defined by **a** immunohistochemistry (IHC) and **b** fluorescence in situ hybridization (FISH)

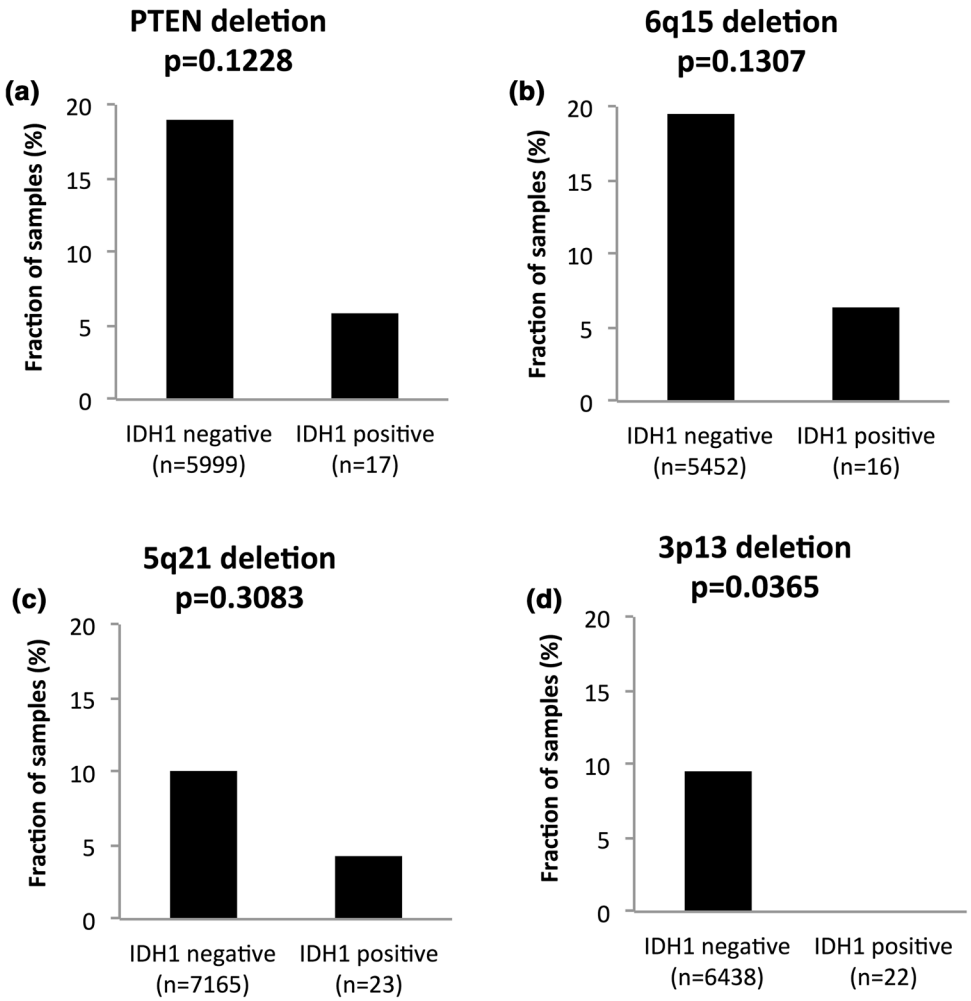

Fig. 4 Association between IDH1^{R132H} staining and 10q23 (*PTEN*), 5q21 (*CHD1*), 6q15 (*MAP3K7*), 3p13 (*FOXP1*) deletion

Discussion

The results of this study demonstrate that IDH1 mutations occur in prostate cancer but at very low frequency, and that they are usually limited to cancer subpopulations. A positive IDH1^{R132H} staining was found in only 42 of 15,531 (0.3%) analyzable prostate cancers in this study. The applied antibody "IDH1^{R132H} clone H09" is a well-established mutation-specific antibody that has shown 94–100% sensitivity and 100% specificity for detection of IDH1^{R132H} mutations [20]. The clear-cut distinction of positive cancers—which were always strongly stained—from negative cancers, which did not show the slightest staining, argues for the quality of the reagent used in this study and the validity of our method. Our findings argue for IDH1^{R132H} mutation rates well below 0.5% in prostate cancer. This fits perfectly with next generation sequencing data generated within The Cancer Genome Atlas (TCGA) and the International Cancer Genome Consortium (ICGC) studies [21]. These groups found IDH1 codon 132 mutations in 11 of 1843 (0.6%) prostate cancers. However, only seven of these 11 mutations were Arg132His, while the remaining four cases had other amino acid substitutions (Arg132Cys and Arg132Gly). These mutations have the same effect as Arg132His but are not identified by the antibody IDH1^{R132H} clone H09 [22, 23]. Higher IDH1 mutation rates had earlier been suggested from 2 studies analyzing cohorts of 118 and 75 cancers. One of these studies employed the same antibody on a TMA and identified positivity in 3 of 118 (2.5%) prostate cancers [24]. The other study used single-strand conformation polymorphism (SSCP) analysis to find a mutation in 2 of 75 (2.7%) prostate cancers from Korean patients [16]. We do not feel that the two previous studies suggesting IDH1 mutation rates greater than 2% really contradict our data as the number of patients analyzed in these studies is too low to assess events occurring in the 0.5–3% range. It cannot be excluded, however, that Korean patients have a higher rate of IDH1 mutations than the mostly Caucasian population analyzed by the ICGC/TCGA consortium and us. There are various examples of ethnic differences in cancer biology. For example, HER2 amplification occurs markedly more frequent in Korean or Arabian than in Caucasian breast cancer patients. Overall, the available data demonstrate that, at least in Caucasian patients, the frequency of IDH1 mutations is about 0.5% in prostate cancer. Our findings in > 15,000 patients further show that IDH1^{R132H} mutations are not strongly linked to a particular tumor phenotype or patient outcome in untreated patients. Of interest, IDH1^{R132H} mutations were predominantly seen in cancers lacking ERG rearrangements, and IDH1^{R132H} positive cancers tended to have lower deletion rates. These data fit well with the findings of the TCGA consortium suggesting that IDH1 mutation defines a distinct molecular subtype of prostate cancers. Based on the comprehensive analysis of 333 prostate cancers, the consortium identified seven distinct molecular subtypes, four of which were characterized by gene fusions involving members of the ETS family of transcription factors (ERG, ETV1, ETV4, and FLI1), and three of which were defined by mutations of the SPOP, FOXA1, and IDH1 genes [17].

The reason for a paucity of molecular aberrations in IDH1-mutated cancers is not obvious. It may, however, be possible that impaired repair efficacy in IDH1-mutated cells may harm particularly such tumor cells that have already acquired a certain degree of genetic instability as indicated by translocations and deletions. In such cases, spontaneous IDH1 mutation may potentiate the risk for accumulating very high—and eventually lethal—numbers of genetic defects. It is thus possible that IDH1 mutations are generally limited to genetically more stable tumor subsets. Of note, these speculations are based on only very few observations.

The detection of IDH1 mutations has potential clinical relevance. Preclinical studies demonstrated that inhibition of mutant IDH1/2 can impair cell growth and promote differentiation in IDH1-mutated glioma and acute myeloid leukemia (AML) cells [25], decrease intracellular 2-HG levels and reverse DNA and histone hypermethylation [26]. At present, numerous clinical studies are recruiting patients with many different cancer types in order to evaluate several drugs targeting mutant IDH1 protein (NCT02746081, NCT02632708, NCT02074839, NCT02073994). Given the high quality of mutation-specific diagnostic antibodies, one could imagine that this mutation results in a highly immunogenic epitope. Diverse vaccines against mutant IDH1/2 have indeed been developed and some of them showed activity in sarcoma and glioma models [27, 28]. In addition, several studies reported better response to chemo- and radiotherapy in IDH1-mutated gliomas [29], possibly as a consequence of altered oxidative stress responses [30]. The high rate of heterogeneity observed for IDH1 mutations may, however, limit therapeutic targeting of these mutations in prostate cancer. It appears very likely that a potential drug or vaccination effect largely depends on whether the entire tumor or only a fraction is IDH1 positive. The analysis of multiple blocks of our prostate cancers for which a positive immunostaining had been detected revealed that all except one IDH1-positive cancers had a heterogeneous mutation status suggesting that IDH1 mutation typically occurs late during tumor progression. Finding one case with homogenous IDH1 mutation, however, indicates that IDH1 mutation can also occur in early stages of the disease—and that anti-IDH1 therapy may be applicable in a very small subset of patients.

In summary, our data show that about 0.3% of prostate cancers have an IDH1^{R132H} mutation and that these are

mostly heterogeneous. Once specific anti-IDH1 therapy becomes reality, only a very small group of prostate cancer patients may benefit from such a treatment.

Author contributions RS, GS, and TS developed the project; AH, MB, MK, CMK, SS, AL, AA, CW, EN, CG, FB, SM, WW, FJ, TSC, TK and MCT collected data; AH, CHM, MK, RS and CS did data analysis; AH, MK, RS, GS and CS wrote the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare they have no conflict of interest.

Ethics approval The study was approved by the ethics committee Ärztekammer Hamburg (WF-049/09 and PV3652). The work has been carried out in compliance with the Helsinki Declaration.

Informed consent According to local laws (HmbKHG, §12,1) informed consent was not necessary.

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