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ORIGINAL ARTICLE

Whole genome and transcriptome amplification: practicable tools for sustainable tissue biobanking?

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Abstract The use of whole genome amplification (WGA) and whole transcriptome amplification (WTA) techniques enables the enrichment of DNA and RNA from very small amounts of tissue. Here, we tested the suitability of WGA and WTA for tumor tissue biobanking. DNA and RNA from 13 standardized and seven non-standardized frozen and 12 formalin-fixed, paraffin-embedded (FFPE) clear cell renal cell carcinoma specimens (>9 years old) served to test the robustness of the WGA and WTA products by reidentifying von Hippel–Lindau (*VHL*) gene mutations known to exist in these samples. The enrichment of DNA and RNA from frozen tissue was up to 1,291-fold and 423-fold, respectively. The sizes and yields (10- to 73-fold) of the amplified DNA obtained from the 12 FFPE samples were generally lower. The quality of the RNA from the FFPE samples was too low to reliably perform WTA. Our results demonstrate that frozen tumor tissue is very suitable for WGA and WTA. All 20 VHL mutations were verified with WGA. Notably, we were able to show that 18 of the 20 (90 %) *VHL* mutations are also transcribed. In FFPE tumor tissue, 8 of 12 cases (67 %) showed the expected mutations after the first WGA. Accurate WTA with FFPE material is sophisticated and strongly depends on the modification and degradation status of the fixed tissue. We conclude that for sustainable tissue biobanking, the use of WGA and WTA is a unique opportunity to provide researchers with sufficient amounts of nucleic acids, preferably from limited frozen tissue material.

Keywords WGA · WTA · Tissue biobank · *VHL* gene · Mutation analysis

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Introduction

Tumor tissue biobanks represent an ideal platform for translational research. Large tumor tissue collections exist in pathology institutes at universities in which thousands of tissue samples are stored. In general, tumor tissue samples are formalin-fixed and paraffin-embedded (FFPE). The volume of archived tissue samples is limited by the size of the paraffin block and varies between a few cubic millimeter (fine needle biopsies) and 1–2 cm^3.

Due to formalin fixation, the molecular structures in FFPE material become modified, degraded, and cross-linked [1] and are of limited value, particularly when high-throughput technologies, such as DNA microarrays, deep sequencing, or mass spectrometry, are to be applied. As a result, frozen tumor tissue samples not required for diagnostic purposes are routinely collected in parallel in many pathology institutes.

According to the recommendations of the TuBAFrost consortium [2], approximately 0.5 cm^3 is the ideal size for collecting frozen tumor tissue samples in a tumor tissue biobank. This is also in line with our standardized operating procedure for freezing and processing tumor tissues which was established at the Central Tissue Biobank at the University Hospital of Zurich [3].

Depending on the organ and the surgical resection specimen that a tumor originated from, residual tissue fragments available for the biobank may be very small. Furthermore, it is expected that ongoing optimization of surgical dissection techniques and cancer screening tests will lead to a further increase of micro-biopsy samples in the biobanks. Subsequently, although the demand for tumor tissue in the cancer research community is rising, the tissue material available for research projects will become more and more limited in the near future. In addition, once tumor samples have been depleted, these samples are no longer available for further

studies. As a consequence, biobank managers are forced to look for suitable strategies to help prevent the wasting of valuable tumor tissue material. The amplification of whole genomes and transcriptomes of tumor tissue samples may be such an approach.

Several companies offer commercially available kits which allow the amplification of the human genome and transcriptome up to over 1,000-fold [4–6]. In this study, we evaluated the suitability of a whole genome amplification (WGA) and a whole transcriptome amplification (WTA) kit for tumor tissue biobanking using tissue from FFPE and frozen clear cell renal cell carcinoma (ccRCC) specimens. The GenomePlex® WGA2 kit (Sigma-Aldrich) is based on the random fragmentation of genomic DNA and conversion of the resulting small fragments to PCR-amplifiable DNA molecules flanked by universal priming sites. In the whole transcriptome amplification kit (WTA2, Sigma-Aldrich), RNA undergoes a single-step conversion into cDNA fragments flanked by universal priming sites followed by a subsequent PCR amplification of the resulting cDNA library using universal oligonucleotide primers. The accuracy and robustness of the WGA and WTA products were tested using RCC samples with known von Hippel–Lindau (*VHL*) mutations [7].

Materials and methods

Tissue and nucleic acid extraction

Twenty frozen and 12 FFPE unrelated ccRCC specimens with known *VHL* mutations [7] were selected for this study to test whether WGA and WTA are applicable to tissues that were treated differently and of a different age. Thirteen of the 20 samples were frozen according to a standardized procedure [3] which was established at our institute in 2006 and were expected to serve as "positive controls". The remaining seven tissues were collected before 2006 using non-standardized protocols. The 12 FFPE ccRCC specimens were between 9 and 19 years of age. The *VHL* mutations are listed in Tables 1 and 2. Hematoxylin and eosin-stained (HE) sections were reviewed by one pathologist (H.M.). All tumor samples contained at least 70 % tumor cells. Magnified HE sections of three tumors are shown in Fig. 1. DNA and RNA were isolated from one and five 20 μm frozen sections, respectively, using the DNeasy Blood and Tissue and the RNeasy Mini Kits (Qiagen, Hilden, Germany), respectively. DNA and RNA from FFPE tissue were isolated from three punched tissue cylinders (diameter 0.6 mm) using the EZ1 DNA Tissue kit (Qiagen) and an RNA extraction protocol [8], respectively. The concentrations of the obtained nucleic acids were measured using the Nanodrop (Thermo Fisher Scientific, Waltham, MA, USA).

Whole genome and transcriptome amplification

The GenomePlex® WGA2 and WTA2 tissue kits (Sigma-Aldrich, St. Louis, MO, USA) were used to amplify the genomes and transcriptomes of both frozen and FFPE tissue. We used between 8 and 38 ng of DNA and RNA from frozen tissue, and 100 ng of DNA from FFPE material as input for the WGA and the WTA, respectively. The quality of the amplified DNA was analyzed on 1.5 % agarose gels. The amplified products were purified using the MinElute PCR Purification Kit (Qiagen), and the concentrations were measured using the Nanodrop.

VHL mutation analysis

PCR of the three *VHL* exons in the amplified genomes was performed as previously described [9]. One hundred nanograms of amplified DNA was used as template.

Using the Primer3 online program, new primers were designed to analyze the *VHL* mutations in the amplified transcriptomes. One primer pair (forward: 5′-gagtacggccct gaagaaga-3′; reverse: 5′-ggcacaaataattcagtttggtt-3′; 350 bp) spanned exons 1 and 2. The second primer pair (forward: 5′-acacgatgggcttctggtta-3′; reverse: 5′-tcaatctcccatccgttgat-3′; 271 bp) spanned exons 2 and 3. One hundred nanograms of amplified cDNA was used as template.

Sequencing of the PCR products was performed by the dideoxy chain-termination method using the BigDye® Terminator v1.1 Cycle Sequencing kit (Applied Biosystems). Forward and reverse primers used for the PCR were also used for sequencing. Cycle sequencing products were analyzed using the AbiPrism 3100 Genetic analyzer (Applied Biosystems).

A second PCR was performed if no amplified product or no *VHL* mutation was obtained. If the result was not in accordance with the expected result, the original DNA or RNA was subjected to a second or third WGA or WTA, respectively. The products underwent *VHL*-PCR and DNA sequencing as described above.

Results

Whole genome amplification

Between 8 and 35 ng of DNA was used as template for the whole genome amplification of frozen ccRCC tissue. The sizes of the resulting PCR products ranged between 100 and 1,000 bp (Fig. 2a). A DNA yield between 2.98 and 17.36 μg of DNA was obtained. Seventeen samples had more than 10 μg of DNA which is equivalent to a 300- to 1,291-fold enrichment. There were no significant differences in the amplified DNA sizes and the yields between tissue samples

Table 1 WGA and WTA results with frozen ccRCC tissue

Year of sample	*VHL* mutation	Starting amount DNA (ng)	DNA yield (μg)	OD 260/280	Fold enrichment	Result	Starting amount RNA (ng)	cDNA yield (μg)	OD 260/280	Fold enrichment	Result
1992	c.492_511del/p.Gln164Glnfs	11	11.40	1.93	1,037	Correct (1st WGA)	15	4.2	2.10	275	Sequence n.a. (1st WTA)
											No mutation (2nd WTA)
2004	c.263_264GG > CT/p.Trp88Ser	19	2.98	1.88	157	Correct (1st WGA)	38	10.1	1.99	267	Correct (1st WTA)
2004	c.400G > T/p.Glu134X	17	5.48	1.89	322	Correct (1st WGA)	30	8.8	1.94	298	No mutation (1st WTA)
											Correct (2nd WTA)
2004	c.300_308del/p.Thr-100_Pro103delinsThr	18	12.11	1.91	673	Correct (1st WGA)	35	7.1	1.94	202	Correct (1st WTA)
2005	c.473T > G/p.Leu158Arg	14	6.06	1.92	433	Correct (1st WGA)	20	7.2	1.96	369	Correct (1st WTA)
2005	c.484T > C/p.Cys162Arg	15	13.27	1.93	885	Correct (1st WGA)	18	6.2	2.11	343	Correct (1st WTA)
2005	c.512delA/p.Lys171Serfs	11	10.32	1.93	938	Correct (1st WGA)	32	9.2	2.00	288	Correct (1st WTA)
2006	c.255_256GC > TA/ p.LeuPro85_86LeuThr	10	11.38	1.94	1,138	Correct (1st WGA)	22	9.1	1.90	423	Correct (1st WTA)
2006	c.234delT/p.Asn78Asnfs	8	10.33	1.92	1,291	Correct (1st WGA)	25	9.5	2.11	374	Correct (1st WTA)
2006	c.232_250del/p.Asn78fs	35	10.49	1.9	300	Correct (1st WGA)	22	4.6	1.98	211	Correct (1st WTA)
2006	c.599_600delGG/p.Arg200fs	26	11.99	1.91	461	No mutation (1st WGA) Correct (2nd WGA)	31	9.7	1.97	309	Correct (1st WTA)
2006	c.562_565del/p.Leu188fs	18	12.00	1.92	667	Correct (1st WGA)	22	8.6	1.95	386	Correct (1st WTA)
2006	c.240T > A/p.Ser80Arg	15	11.22	1.89	748	Correct (1st WGA)	21	6.8	1.96	330	No PCR product (1st WTA)
											Correct (2nd WTA)
2006	c.277G > C/p.Glu93Arg	20	11.21	1.91	561	Correct (1st WGA)	31	8	1.96	255	Correct (1st WTA)
2006	c.208G > T/p.Glu70X	20	11.41	1.9	571	Correct (1st WGA)	31	7.9	1.99	253	No PCR product (1st WTA)
											Correct (2nd WTA)
2007	c.266T > C/p.Leu89Pro	14	14.20	1.98	1,014	No mutation (1st WGA) Correct (2nd WGA)	21	7.3	2.01	340	Correct (1st WTA)
2007	c.421_430dup/p.Gly144Glufs	18	13.26	1.99	737	No mutation (1st WGA)	38	8.9	1.97	237	Sequence n.a. (1st WTA)
						Correct (2nd WGA)					No mutation (2nd WTA)
2007	c.484T > /p.Cys162Arg	19	17.36	1.89	914	No mutation (1st WGA) Correct (2nd WGA)	28	9	2.08	319	Correct (1st WTA)
2007	c.458T > C/p.Leu153Pro	19	13.74	1.96	723	No mutation (1st WGA)	38	11.8	2.02	315	No mutation (1st WTA)
						Correct (2nd WGA)					Correct (2nd WTA)
2007	c.564-565GG > AT/p.LeuGlu188_189LeuX	19	16.63	1.98	875	No mutation (1st WGA)	38	14.9	2.01	397	Sequence n.a. (1st WTA)
						Correct (2nd WGA)					Correct (2nd WTA)

n.a. not analyzable

Table 2 WGA results with FFPE ccRCC tissue

Year of sample	*VHL* mutation	Starting amount DNA (ng)	DNA yield (µg)	OD 260/280	Fold enrichment	Result
1993	c.491_494delAGGT/p.Gln164fs	100	2.89	1.8	29	Correct (1st WGA)
1994	c.257C > A/p.Pro86His	100	1.26	2.01	13	Correct (1st WGA)
1995	c.389T > A/p.Val130Asp	100	1.1	1.71	11	Different mutation (1st WGA)
						No mutation (2nd WGA)
1995	c.472C > G/p.Leu158Val	100	3.19	1.89	32	Correct (1st WGA)
1995	c.374_380del/p.His125fs	100	1.93	1.83	19	Correct+additional mutation (1st WGA)
						Correct (2nd WGA)
1997	c.234T > A/p.Asn78Lys	100	1.04	1.68	10	Correct (1st WGA)
2000	c.468_470delTAC/p.Tyr156	100	6.01	1.85	60	No mutation (1st WGA)
						Correct (2nd WGA)
2000	c.409delG/p.Val137fs	100	4.5	1.86	45	Correct (1st WGA)
2001	c.323insC/p.Arg108fs	100	4.26	1.87	43	Correct (1st WGA)
2002	c.234T > G/p.Asn78Lys	100	3.5	1.78	35	Correct (1st WGA)
2002	c.452T > C/p.Ile151Thr	100	7.26	1.88	73	Correct + additional mutation (1st WGA)
						Correct (2nd WGA)
2003	c.264G > T/p.Trp88Cys	100	2.2	1.78	22	Correct (1st WGA)

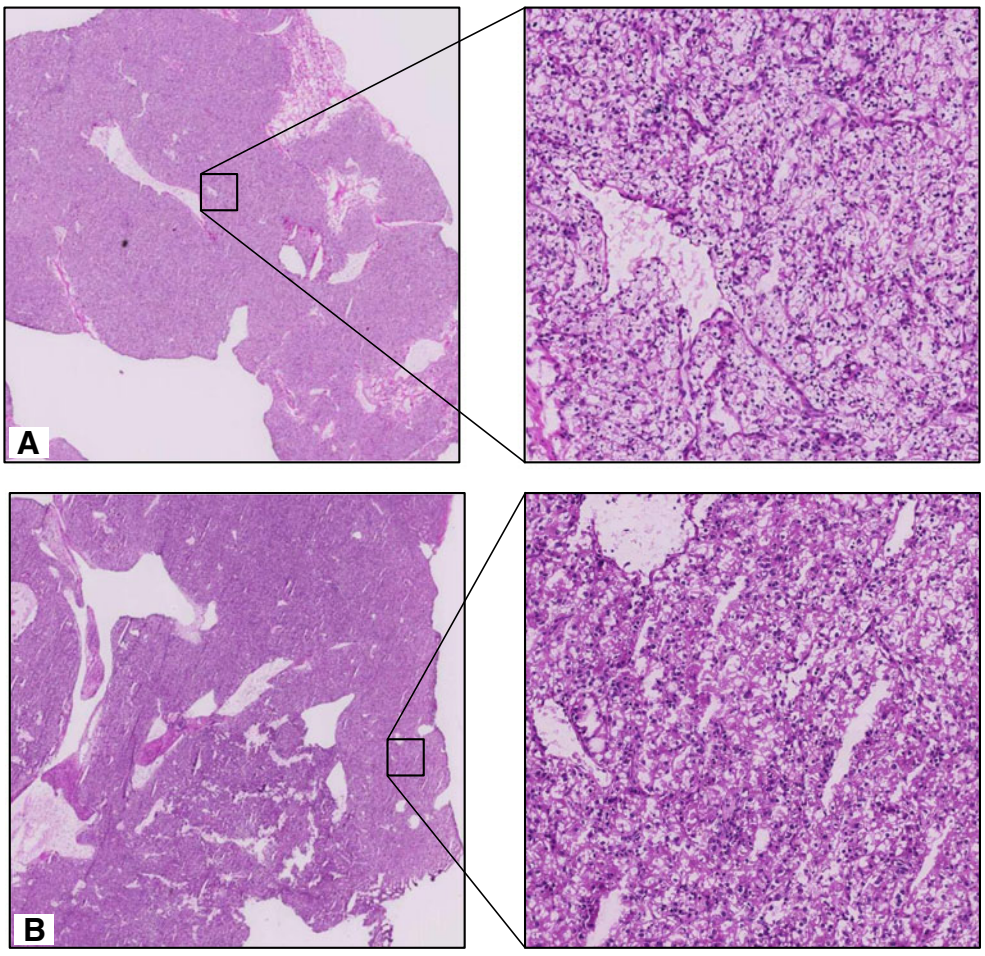

Fig. 1 HE sections of two homogenous ccRCC cases with strongly dominating *VHL* wildtype sequences. Tumors with *VHL* mutations c.458T>C/p.Leu153Pro and c.564-565GG>AT/p.Leu-Glu188-189LeuX, respectively. Details are shown in Table 1

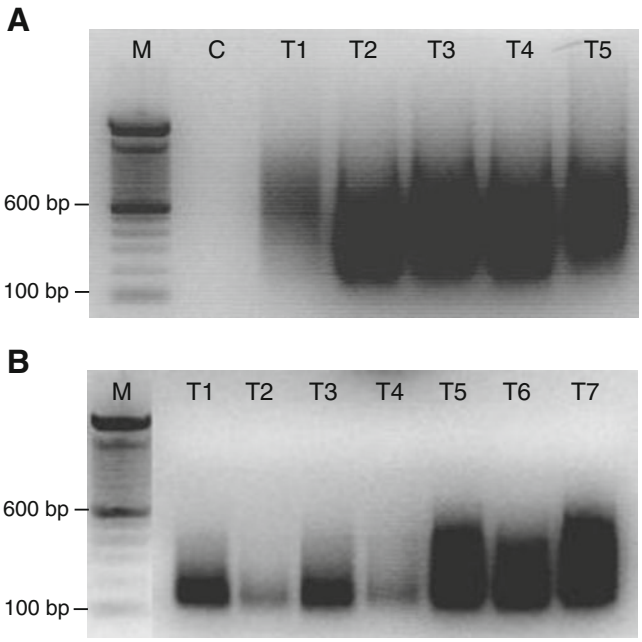

Fig. 2 A 1.5 % agarose gel with WGA products of DNA isolated from five frozen ccRCCs (**a**) and of DNA isolated from seven FFPE ccRCCs (**b**). *M*, 100 bp DNA ladder; *C*, negative control (no DNA template); *T1*, tumor 1, etc

collected under standardized and non-standardized conditions. The detailed results are shown in Table 1.

In contrast to the frozen tissue samples, the sizes and yields of the genomic DNA amplification products obtained from the 12 FFPE ccRCC samples varied significantly among each other and were generally lower (Fig. 2b). As recommended by the supplier, 100 ng of DNA from each of the ccRCC samples was used as input for the whole genome amplification. The DNA yields were between 1.04 and 7.26 μg, which is equivalent to a 10- to 73-fold enrichment. Details are listed in Table 2.

Whole transcriptome amplification

Fifteen to 38 ng of RNA, extracted from the same 20 frozen ccRCC samples, was used for the whole transcriptome amplification. The quality of the amplified cDNA was not analyzed. Between 4.2 and 14.9 μg of amplified cDNA was obtained after the WTA, which is a 202- to 423-fold enrichment. The sizes and yields of amplified cDNA obtained from standardized and non-standardized tissue sets were comparable. The results are shown in Table 1.

We also intended to perform WTA with the same 12 FFPE ccRCC samples used for WGA. However, as we were not able to amplify sufficient amounts of the two PCR products from the original RNA extracts (Fig. 3), particularly the 350 bp fragment which spans the *VHL* exons 1 and 2, we decided to omit this analysis.

VHL mutation analysis of WGA products

The WGA products served as DNA templates for *VHL* exon-specific PCRs. For each sample, only the primer pair covering the known mutated exon was used. Fourteen of the 20 (70 %) amplified DNA samples showed the correct mutation after the first WGA. The WGA of six samples using the original DNA preparation from tissues was repeated because the expected *VHL* mutation was not visible in the DNA sequence. However, it is important to note that in the original DNA of these six ccRCC cases, the *VHL* wild-type sequence was strongly dominating and the mutated sequence was underrepresented. The *VHL* mutations of two tumors are illustrated in Figs. 4 and 5. The results of the WGA are shown in Table 1.

The genomes of all of the 12 ccRCC FFPE samples were successfully amplified. After the first WGA, eight (67 %) of these showed the expected mutations. Two cases showed the expected mutations and an additional *VHL* mutation. Of the last two cases, one had no mutation and the other had a different *VHL* mutation. After repeating the WGA with the original DNA samples of the four tumors that showed discrepant results, three had the correct mutation and one had no mutation. The results are shown in Table 2. An example of a *VHL* mutation identified in the original DNA and after WGA of one ccRCC is shown in Fig. 4a and b, respectively.

VHL mutation analysis of WTA products

To see whether a *VHL* gene affected with a mutation is also transcribed in ccRCC, the WTA products of the 20 frozen ccRCC were subjected to a PCR which specifically amplifies *VHL* expressed alleles. Surprisingly, 13 of 20 (65 %) *VHL* mutations were confirmed as transcribed after the first WTA and five (25 %) after the second PCR or the second WTA. Six of the 20 analyzed ccRCCs had frameshift mutations which probably lead to a loss of function of the VHL protein (pVHL). The frameshift mutations identified in the genomic DNA of the two ccRCC samples could not be verified in the corresponding WTA products. The transcribed *VHL* mutations of two tumors are shown in Figs. 4c and 5c. The results are listed in Table 1.

Discussion

In this study, we demonstrate that WGA and WTA are well suited to sufficiently and accurately enrich DNA and RNA from frozen tumor tissue. The whole amplification of genomes and transcriptomes of nucleic acids extracted from FFPE tumor tissues, however, has limitations. Below, we address some critical points which are of general importance if these methods are used to amplify DNA and RNA from

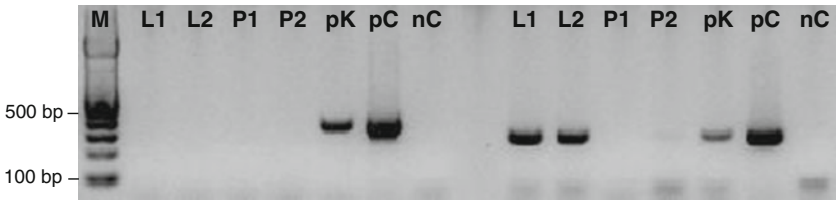

Fig. 3 Reverse transcription-PCR analysis of the 350 bp (exon 1/2) and the 271 bp (exon 2/3) *VHL* fragments. *M*, 100 bp DNA ladder; *L1* and *L2*, FFPE normal liver; *P1* and *P2*, FFPE normal prostate; *pK*, WTA amplified cDNA control from frozen normal kidney; *pC*, plasmid containing the whole *VHL* coding sequence (positive control); *nC*, negative control (no cDNA template)

cancer tissue. For tumor tissue biobanking, these technologies may help to permanently provide sufficient amounts of high quality nucleic acids from limited and valuable tumor tissue material for research purposes.

Mutated *VHL* alleles are commonly transcribed in ccRCC

It is widely accepted that in ccRCC, *VHL* inactivation follows the two-hit mechanism with loss of the chromosomal 3p arm and a mutation of *VHL* in the remaining allele. Recently, we showed that *VHL* mutations may exert different impacts on pVHL's functionality [7] provided that the mutated gene is expressed. Several data exist about pVHL expression in ccRCC [10–15], but the expression status of mutated *VHL* alleles has not been reported to date. Here, we were able to demonstrate that most of the *VHL* mutated alleles are transcribed, regardless of their mutation type. It is, consequently, conceivable that the location and the type

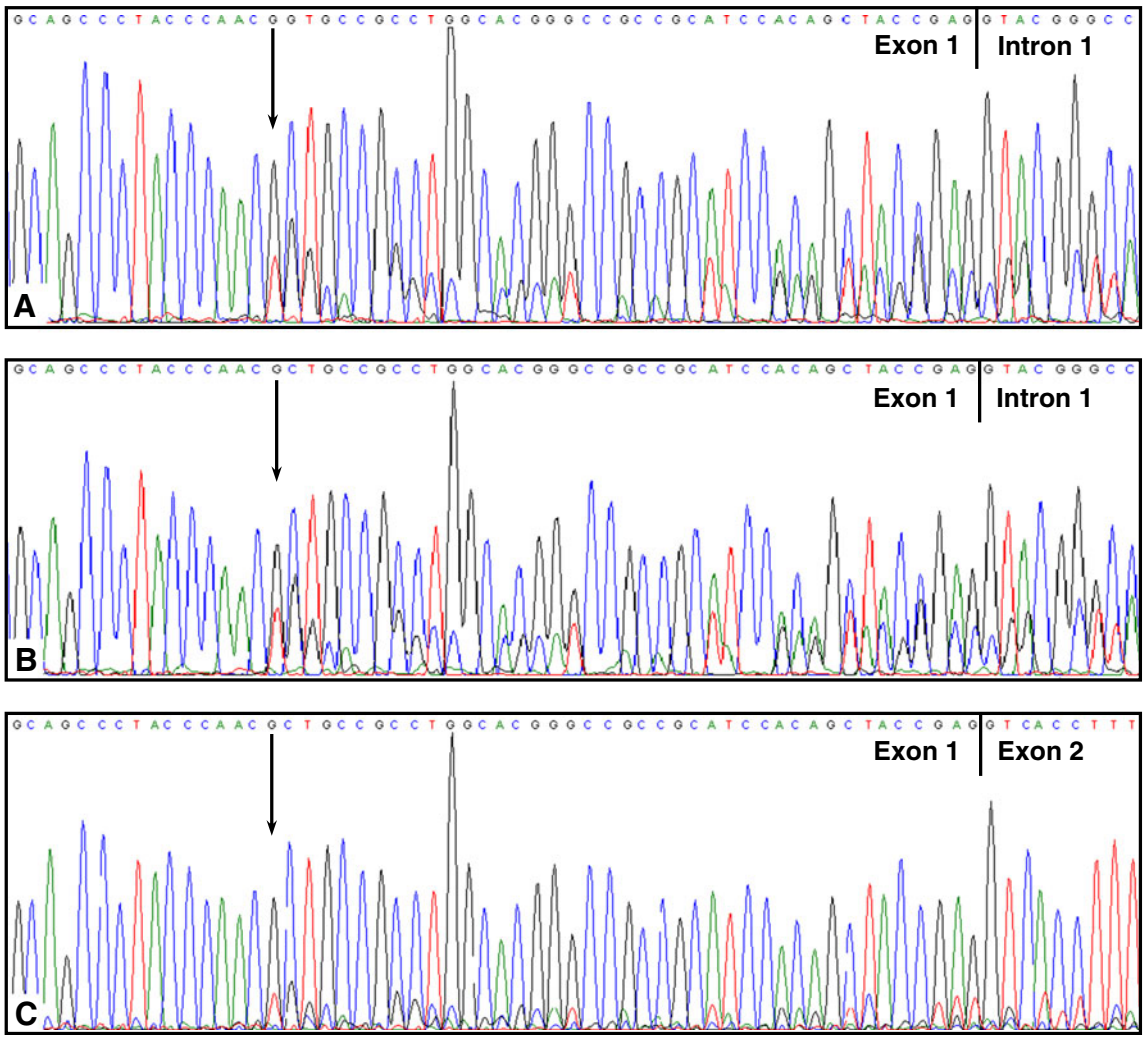

Fig. 4 *VHL* mutation c.300_308del/p.Thr100_Pro103delinsThr in the original DNA of one ccRCC patient (**a**), after WGA (**b**), and after WTA (**c**). The mutation site is denoted by an *arrow*. The boundaries between exon 1/intron 1 and exon 1/exon 2 are indicated

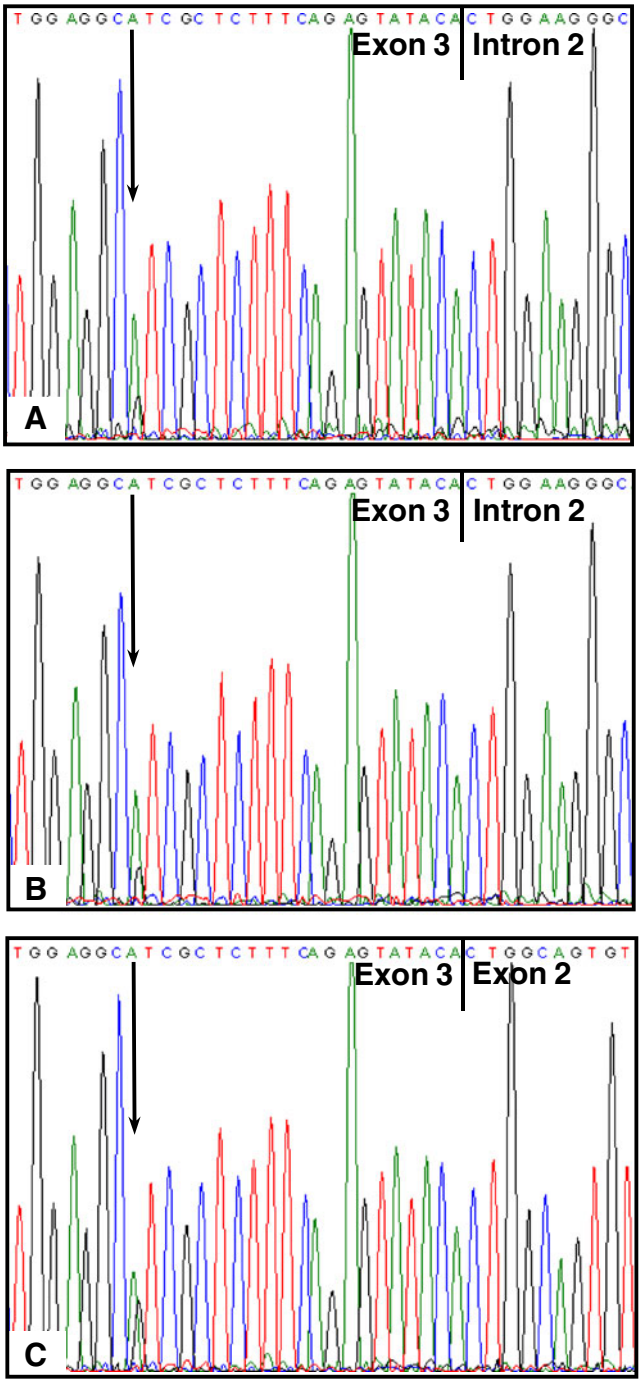

Fig. 5 *VHL* mutation c.484T>C/p.Cys162Arg in the original DNA of one ccRCC patient (**a**), after WGA (**b**), and after WTA (**c**). The sequence is shown in reverse and the mutation site is denoted by an *arrow*. The boundaries between exon 3/intron 2 and exon 3/exon 2 are indicated

of a *VHL* mutation may have dramatic effects on the multi-adaptor functions of pVHL [16] as well as on tumor behavior and response to targeted therapies. Although evidence of mutant pVHL expression in ccRCC has not been reported, previous immunohistochemistry data indicate the presence of pVHL in the majority of ccRCC [10–15].

WGA of DNA extracted from frozen tumor tissue

The DNA obtained after WGA was up to 1,300-fold enriched which is in the range described by the supplier. After the first WGA, the expected *VHL* mutation was present in 14 of 20 samples but absent in six samples. After repeating the WGA from original tissue DNA, all six cases showed the correct *VHL* mutation. In all 20 samples, even in the six ccRCC cases in which the *VHL* mutated sequences were significantly underrepresented, the sizes of the resulting sequence peaks of both the wild-type and the mutated *VHL* sequences obtained after WGA were comparable with those of the original, unamplified DNA (see Fig. 4). As the tumor specimen used for the WGA and sequence analyses consisted of more than 70 % tumor cells, we believe that the observed underrepresention of *VHL* mutations in the six ccRCC is a matter of genetic heterogeneity of the tumor cells. Intratumoral heterogeneity was described in a former study in which *VHL* deletions were analyzed [17]. We conclude that in tumor types that are more heterogeneous than RCC, underrepresented gene sequence alterations may frequently be missed after WGA if whole sections are used for DNA extraction. To minimize this problem, DNA should be extracted from micro-dissected or punched tumor areas rather than from whole tumor sections.

WGA of DNA extracted from FFPE tumor tissue

Although the starting amount of DNA extracted from FFPE ccRCC tissue was 100 ng, the enrichment of DNA was about tenfold lower when compared to WGA with DNA extracted from frozen tumor tissue. WGA with 10 ng of DNA resulted in significantly less amplification product (data not shown). Two-thirds of the samples had the correct mutation after the first WGA. Discrepant results were obtained from the four remaining cases that had either different, additional, or no *VHL* mutations. After a second, separate WGA, three of these cases showed the correct mutation and only one case (with a different mutation after the first WGA) had no mutation. It is known that the frequency of damaged bases can vary considerably due to the fixation time and the modifying effects of formalin on DNA in tissue [18, 19]. Therefore, the results obtained from a mutation analysis should be interpreted with care. In a previous comprehensive *VHL* mutation study, we could not validate the results from approximately 10 % of the formalin-fixed ccRCC samples [7]. At least two independent rounds of PCR and sequencing were performed to distinguish between real and artificial mutations.

A previous study showed that FFPE tissue is less accurate than frozen material for determining mutations in tumors [20]. If WGA is to be performed with DNA from FFPE tumor material, we suggest that two separate parallel WGA

rounds using DNA from original tissue are carried out to select bad from good quality tissue specimens. As a quality control of the whole genome amplified DNA, any exonic sequence can be PCR-amplified and sequenced. If the two obtained sequences are well analyzable and identical, then the integrity of the WGA DNA is given. In case of a discrepancy between the sequences, a third WGA, PCR, and sequencing analysis should be included and compared with the first two sequences. If this third WGA sequence corresponds to one of the others, these two may be used for further molecular analyses. If, however, three different results are obtained, this tissue sample should not be considered for further analyses. The proposed strategy for testing the quality of WGA products is schematically illustrated in Fig. 6.

To further minimize PCR artifacts, the use of non-cross-linking fixatives may be an alternative to buffered formalin as the quality of the nucleic acids seems to be similar to those derived from frozen tissue [21, 22]. A further option may be to treat DNA isolated from FFPE tissue with uracil-DNA glycosylase to reduce uracil lesions which are the major cause of sequence artifacts [23].

WTA of RNA extracted from frozen tumor tissue

The enrichment of amplified cDNA varied only between 200- and 400-fold, indicating a uniform WTA of all 20 RNA samples. With the exception of two ccRCC samples, which had *VHL* frameshift mutations in the original DNA, the expected *VHL* mutations were found to be transcribed in

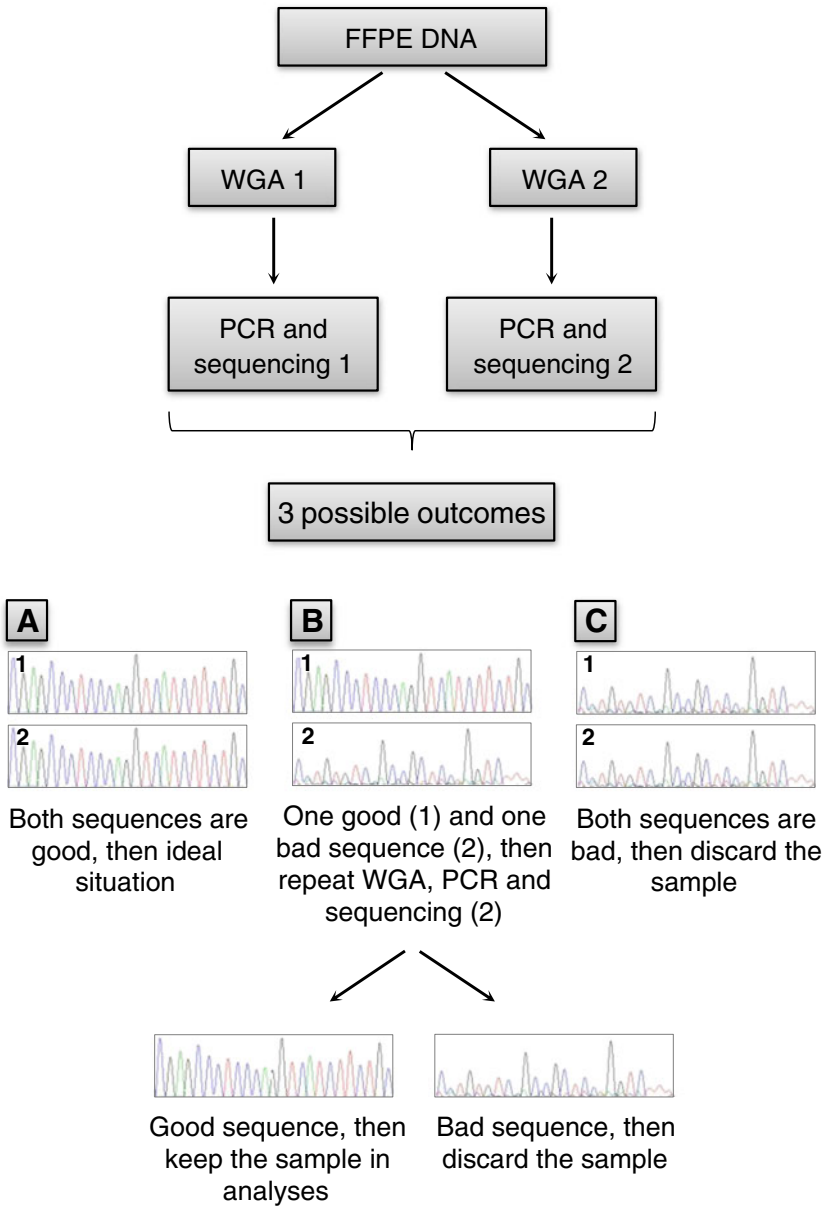

Fig. 6 Proposed strategy to test the quality of DNA from WGA products derived from FFPE tissue. The original DNA is used for additional WGA rounds

the remaining 18 ccRCC samples. To our knowledge, there are no studies to date demonstrating that *VHL* mutations are expressed at the RNA level in sporadic ccRCC, regardless of the mutation type.

In 7 of 20 cases, the *VHL* mutation could not be evaluated after the first WTA. After repeating the WTA, the *VHL* mutations were found in five cases but were not visible in two samples. Not only technical problems (no PCR product of the *VHL* fragment or non-analyzable sequence) but also *VHL* mutations, which can lead to the downregulation or even to the loss of RNA expression, may explain the discrepant findings. These results demonstrate that whole transcriptomes from frozen tissues are linearly amplified and are applicable for gene expression, specific gene mutation, as well as RNA-Seq analyses.

WTA of RNA extracted from FFPE tumor tissue

As already outlined for DNA, the quality of the RNA extracted from FFPE material is similarly low and, therefore, of limited value for molecular analysis [24]. The amplification of sequences becomes increasingly problematic with the size of the PCR product. To ensure that only cDNA was being amplified, we worked with primer pairs that spanned two *VHL* exons. The sizes of the PCR fragments were, however, too large to amplify sufficient amounts of DNA for sequence analysis. As it was suggested in other studies [1, 25, 26], our results imply that a successful WTA-based mutation analysis of FFPE tissue is strongly dependent on the chosen size of the PCR fragment to be analyzed and the age of the tissue sample.

WGA and WTA for economic tumor tissue biobanking?

WGA and WTA technologies can help to provide sufficient amounts of high quality nucleic acids from valuable tumor tissue material for research purposes in tumor tissue biobanking. By using fixed and non-fixed ccRCC nucleic acids with known *VHL* mutations, we demonstrate that nucleic acids from frozen tumor tissue are most suitable for WGA and WTA. The severity of the modification and degradation of nucleic acids in FFPE tumor tissue often differs and thus hampers the accurate amplification. Additional rounds of WGA or WTA and validation experiments lead to increased costs. In Switzerland, one WGA costs approximately 10 Swiss Francs and one WTA is about eight times more expensive. The routine use of WGA and WTA for large tumor tissue biobanks, which receive thousands of tissue samples annually, would cause an enormous financial burden on a biobank's budget, in terms of manpower, consumables, freezers, space, and logistics. In contrast, research projects usually focus on analyzing a few dozen up to several hundred tissue samples of one specific tumor type, which represents only a tiny part of the whole inventory of a large tumor tissue biobank. The use of WGA and WTA should be restricted to those tissue samples, preferably frozen ones, required for approved research projects. This would help to keep the costs within an affordable limit. Furthermore, to financially fund tumor tissue biobanks, it will be of utmost importance for cancer research scientists to include costs for tissue biobanking, such as sampling, storing, and processing as well as the costs for WGA and WTA in grant applications if they intend to work with DNA and RNA from cancer tissue.

Conclusions

Our data indicate that both WGA and WTA are feasible with nucleic acids extracted from frozen and FFPE tissue. Nevertheless, the use of the latter has certain limitations and the results should be interpreted with caution. Although standardized procedures are required to guarantee the high quality of frozen tissue collections, it seems that non-standardized protocols formerly used for snap freezing native tissue samples do not negatively influence the outcome of WGA and WTA.

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Conflict of interest There is no conflict of interest to declare.

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