p53 overexpression is a prognosticator of poor outcome in esophageal cancer

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Abstract. Immunohistochemistry studies on p53 inactivation in esophageal cancer are available with inconclusive results. Data on the combined effect of p53 protein accumulation and *TP53* genomic deactivation in large scale studies for esophageal cancer are currently lacking. A tissue microarray with 691 esophageal cancer samples was analyzed by p53 immunohistochemistry and fluorescence *in situ* hybridization (FISH). Nuclear p53 accumulation was observed in 45.9% of patients with adenocarcinoma (AC) and in 40.0% in squamous cell carcinoma (SCC). Heterozygous *TP53* deletions occurred in 40.9% in AC and in 19.4% in SCC. Homozygous deletions did not occur at all. High-level p53 immunostaining was associated with shortened overall survival in AC and SCC while *TP53* deletions alone showed no correlation with survival. High-level p53 immunostaining in patients with AC was associated with advanced tumor (P=0.019) and Union for International Cancer Control stages (P=0.004), grading (P=0.027) and the resection margin status (P=0.006). Associations between p53 immunostaining and SCC were not found. *TP53* deletions were found to be associated with advanced tumor stages (P=0.028) and the presence of lymph node metastasis (P=0.009) in SCC. In conclusion, strong p53 immunostaining, but not *TP53* deletion alone, is associated with unfavorable outcomes and may therefore represent a clinically useful molecular marker in esophageal cancer.

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Introduction

Esophageal cancer (EC) remains the sixth most common cause of cancer related death in the world despite recent progress in multimodal therapy concepts involving neoadjuvant therapy and standardized surgical approaches (1). EC is associated with high malignant potential for local invasion and early dissemination resulting in low overall-survival (OS) rates in patients even after curatively intended surgery (2).

There are two major histological types of EC that account for >90% of all malignant neoplasms in the esophagus: squamous cell carcinoma and adenocarcinoma. The pathogenesis in adenocarcinoma is generally considered to be driven by epithelial metaplasia, often caused by acid reflux. In squamous cell carcinoma, malignant transformation is predominantly associated with alcohol intake and smoking (3). The incidence of the particular subtypes has changed in the past years with increase of adenocarcinoma and decrease of squamous cell carcinoma in Western countries, mainly due to life style changes (2). Due to the limited prognosis of the majority of patients, identification of new biomarkers predicting the individual prognosis and response to therapy are of imperative need for allocation of individualized therapy strategies.

The *TP53* gene has been known as one of the most important tumor suppressor genes, located on human chromosome 17p13.1. p53 plays a major role in tumorigenesis as it controls cell growth, apoptosis and regulation of angiogenesis (4). Mutations in *TP53* were found in >50% of human cancers, which makes it one of the most mutated genes in tumors (5). In esophageal cancer, mutations have been described in frequencies between 40-70%, depending on the underlying cell type (4,6-8). As shown for several tumor entities, inactivation of the *TP53* tumor suppressor gene leads to the development of malignant cell clones and accelerates the carcinogenesis (9). Different mechanisms of functional p53 inactivation have been described including functionally relevant point mutations and gross chromosomal alterations, mostly driven by chromosome 17p deletions.

Previous studies reported inconclusive results on whether p53 accumulation has a functional impact on progression of

EC (10-19). While some studies found associations between the p53 expression level and tumor progression or overall-survival, other studies were not able to confirm these findings. Data on *TP53* deletion status is available from only one report including 40 patients suffering from esophageal squamous cell carcinoma (20).

To further elucidate the clinical relevance of TP53 mutations and p53 expression as prognostic biomarkers in esophageal cancer we took advantage of our preexisting tissue microarray (TMA) containing nearly 700 esophageal cancer specimens with attached clinical follow-up and clinico-pathological data.

Our findings demonstrate that strong p53 immunostaining is correlated with unfavorable prognosis in esophageal cancer, while homozygous *TP53* deletions represent a catastrophic event leading to cell death.

Materials and methods

Patients. For this study, specimens from patients that had undergone tumor resection in curative intent between 1992 and 2014 at the University Medical Center, Hamburg-Eppendorf were included. Tissue samples from 691 patients were analyzed including 398 esophageal adenocarcinomas and 293 squamous cell carcinomas. All data including patient sex, tumor histology, size, lymph node metastasis and disease stage (UICC 7th edition) were obtained by reviewing a combination of clinical and pathological records, outpatient clinic medical records, epidemiological cancer surveillance data bases and by communication with the patients and their attending physicians. All resections were performed as en-bloc esophagectomies with radical two field lymph node dissection. Fifty patients underwent neoadjuvant therapy (AC n=30, SCC n=20). Patients that died within 30 days due to postoperative complications were not considered for survival analysis.

Informed consent was not required due to the retrospective nature this study. Analysis of anonymized human tissue samples by the treating physician (including the pathologist) is permitted according to local laws (§12a Hamburgisches Krankenhausgesetz). In addition, we obtained approval for manufacturing and analyzing tissue microarrays made from tissue samples from anonymized donors from our local review board, the Ethics Commission of the Ärztekammer Hamburg (no. WF049/09).

TMA construction. The TMA was constructed as previously described (21). In brief, tissue cores were obtained from formalin-fixed paraffin-embedded (FFPE) tissue blocks from patients with pathologically proven esophageal cancer. Representative areas of the tumor were selected based on hematoxylin-eosin staining. 691 tissue cylinders with a diameter of 0.6 mm were punched from the 'donor' tissue blocks using a custom-made semi-automatic robotic precision instrument and placed into one empty recipient paraffin block. The resulting TMA blocks were used to produce 4 μm sections that were transferred to an adhesive-coated slide system (Instrumedics Inc., Hackensack, NJ, USA).

Immunohistochemistry. Freshly cut TMA sections were immunostained on one day and in one experiment. Slides were deparaffinized and exposed to heat-induced antigen retrieval for 5 min in an autoclave at 121˚C in pH 7.8 Tris-EDTA-Citrate buffer. Primary antibody specific for p53 (DO1, murine monoclonal IgG_{2a}, Oncogene, Cambridge, MA; USA; dilution 1:3,600) was applied at 37˚C for 60 min. Bound antibody was then visualized using the EnVision Kit (Dako, Glostrup, Denmark) according to the manufacturer's directions.

Colon cancers with known p53 alterations served as positive controls and normal prostate tissue as negative controls on each TMA section. Nuclear p53 staining intensity was scored in a four-step scale (0, 1+, 2+, 3+ staining intensity) and the percentage of stained nuclei was estimated. A final immunohistochemistry (IHC) result was assigned to each tumor as described in earlier studies from our group (22). Negative: No staining at all or 1+/2+ staining in <10% of tumor cells, low: 1+ staining in ≥10% or 2+ staining in ≥10% but ≤70% of tumor cells or 3+ staining in ≤10% of tumor cells, high: 2+ staining in >70% of tumor cells or 3+ staining in >10% of tumor cells. For calculation of results low and high staining intensities were summed up as positive.

FISH. FISH was used to identify genomic *TP53* deletions and translocations. A dual color FISH probe was constructed from a spectrum green labeled BAC clone (BACs RP11-89D11, RP11-404G1; Source Bioscience, Nottingham, UK) and a commercial spectrum orange labeled centromere 17 (CEP17) reference probe (no. 06J36-06; Abbott Molecular, Wiesbaden, Germany). Freshly cut 4 μm TMA sections were used for dual color FISH. Before hybridization, sections were deparaffinized and proteolytically pretreated with a commercial kit (paraffin pretreatment reagent kit; Abbott Molecular), followed by dehydration in 70, 80 and 96% ethanol, air drying and denaturation for 10 min at 72˚C in 70% formamide-23 saline-sodium citrate (SSC) solution. Hybridization was done overnight at 37˚C in a humidified chamber, slides were washed and counterstained with 0.2 mmol/l 40-6-diamidino-2-phenylindole in an antifade solution.

FISH Scoring. Each TMA spot was carefully evaluated and the predominant *TP53* signal counts were recorded for each FISH probe. Tissue samples with missing tumor tissue as determined by corresponding H&E slides were not considered for analysis. In addition, tumor spots were excluded from analysis if there was evidence for insufficient hybridization such as lack of *TP53* signals in both tumor and peritumoral non-malignant tissue. Deletion of *TP53* was defined as presence of fewer *TP53* signals than centromere 17 probe signals (heterozygous deletion) or complete absence of *TP53* signals but presence of at least one centromere 17 probe signal (homozygous deletion) in ≥60% of tumor nuclei. The aforementioned cut-off level was chosen because a good correlation between FISH and array genomic hybridization was already shown by our group for *PTEN* and p53 in prostate cancer (22,23).

Statistical analysis. SPSS Statistics for Mac (version 17; SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. Interdependence between immunostaining and FISH results as well as clinical data was calculated using the Chi-squared and Fisher's exact tests and displayed by cross-tables. Group differences were examined using the t-test. Survival curves

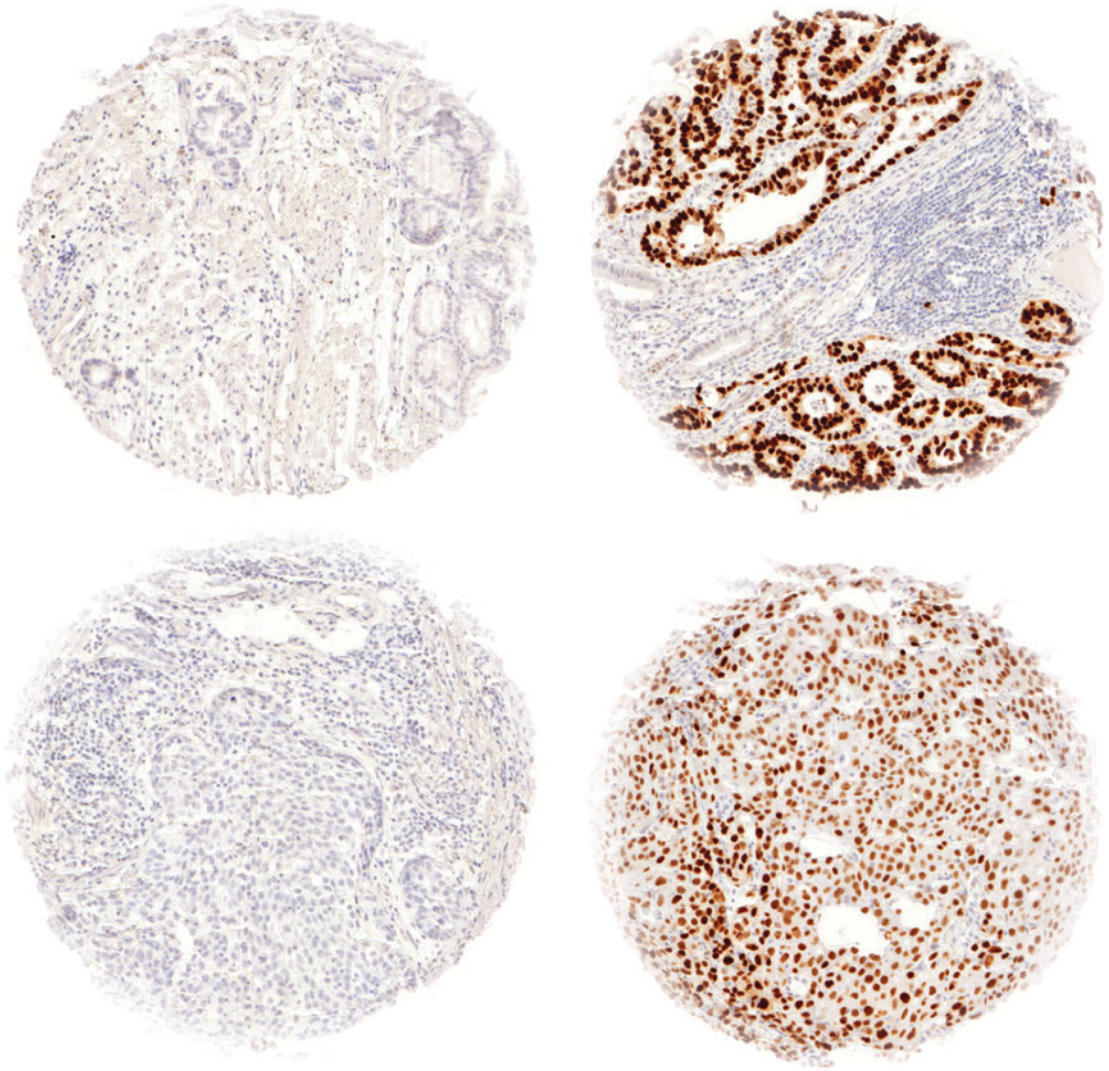

Figure 1. Representative images of negative and positive p53 immunostaining in adenocarcinoma and squamous cell carcinoma.

were plotted using the Kaplan-Meier method and analyzed using the log-rank test. Univariate and multi-variate analyses were performed for prognostic factors of recurrence-free and overall survival using the Cox regression model. All tests were two-sided. $P<0.05$ was considered to indicate a statistically significant difference.

Results

p53 IHC. p53 immunostaining was interpretable in 574 (83.2%) of 691 samples, in total 314 esophageal adenocarcinomas (AC) and 260 squamous cell carcinomas (SCC) were evaluable. Reasons for non-informative cases included lack of tissue samples or absence of unequivocal cancer tissue in the TMA spot. p53 positivity was seen in 104 SCC (40.0%) and in 144 AC of the esophagus (45.9%). Representative images of p53 immunostaining in AC and SCC are given in Fig. 1. p53 positivity was associated with tumor stage (P=0.019), UICC stage (P=0.004), grading (P=0.027) and surgical resection margin status (P=0.006) in esophageal AC (Table I), while no associations between clinico-pathological data and p53 immunostaining in squamous cell carcinoma were revealed (Table II).

TP53 FISH analysis. FISH was interpretable for *TP53* deletions in 269 (67.6%) samples in esophageal adenocarcinoma and 237 (80.9%) samples in squamous cell carcinoma. Non-informative cases were caused by inefficient hybridization, missing tissue spots or absence of representative tumor tissue on the TMA spot. Heterozygous *TP53* deletions were detectable in 110 samples (40.9%) in EC and 46 samples (19.4%) in SCC. *TP53* deletions were associated with age (P=0.031) and surgical resection margin (P=0.029) in adenocarcinoma (Table I). For squamous cell carcinoma, associations were detected between heterozygous *TP53* deletions and tumor stage (P=0.028) and presence of lymph node metastasis (P=0.009) (Table II). Not one single TMA spot revealed cells with homozygous *TP53* deletions.

Combined effect of p53 immunostaining and TP53 deletions. Data on both p53 immunostaining and *TP53* FISH were available from 244 adenocarcinomas and 223 squamous cell carcinomas. A correlation between high p53 immunostaining and *TP53* deletion could not be revealed for AC (P=0.643) while in SCC an association (P=0.044) was seen. In adenocarcinoma, 54 (21.8%) tumors showed heterozygous *TP53*

Table I. Association between p53 immunostaining and *TP53* FISH with clinicopathological parameters in adenocarcinoma.

	p53 IHC result				*TP53* FISH result			
Parameter	Evaluable cases (n)	Negative (%)	Positive (%)	P-value	Evaluable cases (n)	no del (%)	het del (%)	P-value
All tumors	314	54.1	45.9		269	59.1	40.9	
Age, years				0.645				0.031
≤65	107	52.3	47.7		91	89.2	10.8	
>65	207	55.1	44.3		178	69.9	30.1	
Sex				0.186				0.074
Male	262	52.7	47.3		226	63.4	36.6	
Female	51	62.7	37.3		42	95.9	4.5	
Tumor stage				0.019				0.195
pT1	61	70.5	29.5		40	92.2	7.8	
pT2	33	60.6	39.4		26	95.9	4.1	
pT3	96	49.0	51.0		156	72.8	27.2	
pT4	22	45.4	54.6		21	98.1	1.9	
Lymph node metastasis				0.069				0.923
pN0	96	64.6	35.4		79	88.4	11.6	
pN1	51	53.0	47.0		50	92.5	7.5	
pN2	81	51.9	48.1		65	89.2	10.8	
pN3	84	45.2	54.8		74	88.8	11.2	
Distant metastasis				0.475				0.974
M0	277	54.9	45.1		237	63.9	36.1	
M1	37	48.6	51.4		32	95.2	4.8	
UICC stage				0.004				0.977
I	62	72.6	27.4		52	92.5	7.5	
II	41	61.0	39.0		33	95.1	4.9	
III	173	48.0	52.0		151	76.3	23.7	
IV	34	44.1	55.9		30	95.5	4.5	
Grading				0.027				0.922
G1	15	86.7	13.3		12	98.5	1.5	
G2	113	54.9	45.1		98	84.2	15.8	
G3	175	50.9	49.1		152	76.2	23.8	
G4	6	83.3	16.7		3	99.6	0.4	
Surgical resection margin				0.006				0.029
R0	228	59.2	40.8		174	72.7	27.3	
R1	78	38.5	61.5		63	86.4	13.6	
R2	3	66.7	33.3		3	99.6	0.4	

FISH, fluorescence *in situ* hybridization; IHC, immunohistochemistry; UICC, Union for International Cancer Control; het del, heterozygous deletion; no del, no deletion.

deletion accompanied by high p53 expression which was also displayed by 25 SCC (11.2%). However, this finding was merely associated with resection margin status (P=0.013) in esophageal AC.

Survival analysis. In total, 283 patients with adenocarcinoma and 231 with squamous cell carcinoma were available for survival analysis. High-level p53 immunostaining was linked to shortened overall-survival (OS) in both esophageal AC and SCC as analyzed by Kaplan-Meier (P=0.021 and P=0.013, respectively; Fig. 2A and D). *TP53* deletions were not associated with OS irrespective of the histological type (P=0.973 (AC) and P=0.099 (SCC); Fig. 2B and E). Combination of p53 expression and *TP53* deletion status did not improve the prognostic power compared to p53 IHC alone (Fig. 2C and F).

Multivariate analysis. For both histological types, UICC stage and a complete surgical resection (R0) proved to be independent

Table II. Association of p53 immunostaining and *TP53* fluorescence *in situ* hybridization with clinicopathological parameters in squamous cell carcinoma.

	p53 IHC result				*TP53* FISH result			
Parameter	Evaluable cases (n)	Negative (%)	Positive (%)	P-value	Evaluable cases (n)	no del (%)	het del (%)	P-value
All tumors	260	60.0	40.0		237	80.6	19.4	
Age, years				0.678				0.11
<65	101	58.4	41.6		94	90.3	9.7	
>65	159	61.0	39.0		143	90.3	9.7	
Sex				0.568				0.945
Male	190	61.1	38.9		171	86.1	13.9	
Female	70	57.1	42.9		66	94.5	5.5	
Tumor stage				0.694				0.028
pT1	43	67.4	32.6		42	97.9	2.1	
pT2	55	56.4	43.6		46	98.3	1.7	
pT3	144	59.7	40.3		134	85.2	14.8	
pT4	18	55.6	44.4		15	99.2	0.8	
Lymph node metastasis				0.276				0.009
pN0	123	53.7	46.3		115	92.4	7.6	
pN1	58	63.8	36.2		53	95.8	4.2	
pN2	52	67.3	32.7		49	95.8	4.2	
pN3	26	65.4	34.6		20	96.6	3.4	
Distant metastasis				0.873				0.62
M0	211	59.7	40.3		195	83.5	16.5	
M1	49	61.2	38.8		42	97.0	3.0	
UICC stage				0.903				0.074
I	60	13.1	10.0		6	97.5	2.5	
II	60	14.6	8.5		9	96.2	3.8	
III	91	20.8	14.2		23	90.3	9.7	
IV	49	11.2	7.3		8	96.6	3.4	
Grading				0.63				0.81
G1	3	0.4	0.8		4	99.6	0.4	
G2	163	38.1	24.6		151	88.6	11.4	
G3	93	21.5	14.2		81	92.8	7.2	
Surgical resection margin				0.84				0.54
R0	192	43.8	30.0		179	86.4	13.6	
R1	52	12.7	7.3		45	95.3	4.7	
R2	14	3.1	2.3		12	98.7	1.3	

FISH, fluorescence *in situ* hybridization; IHC, immunohistochemistry; UICC, Union for International Cancer Control; het del, heterozygous deletion; no del, no deletion.

prognostic markers as analyzed by multivariate cox-regression model. Furthermore, strong p53 immunostaining was also independently associated with OS in SCC (Table III).

Discussion

Our study shows that p53 overexpression is linked to shortened overall survival in patients suffering from EC. To our knowledge, this is the study includes the largest number of cases for EC and SCC with corresponding survival data. Furthermore, we correlated p53 IHC and TP53 FISH analysis. As follows in the discussion, previous studies either present inconclusive results or include only few patients.

In our analysis p53 alterations are present in 40-45% of esophageal cancer, irrespective of the underlying histological type. Genome studies conducted either with array comparative genome hybridization (CGH) or whole genome sequencing repeatedly revealed *TP53* mutations as the most

Table III. Multivariate analysis of p53 immunostaining in esophageal cancer.

	Adenocarcinoma				Squamous cell carcinoma			
		95% confidence interval				95% confidence interval		
Parameter	HR	Lower	Upper	P-value	HR	Lower	Upper	P-value
Sex (male/female)	0.741	0.462	1.187	0.212	0.815	0.562	1.184	0.284
Age group (<65/>65 years)	1.414	0.965	2.072	0.076	0.920	0.663	1.277	0.620
UICC Stage	2.232	1.626	3.065	<0.001	1.288	1.019	1.629	0.034
Distant metastasis	1.095	0.581	2.063	0.780	1.001	0.579	1.730	0.998
Surgical resection margin	1.677	1.152	2.440	0.007	1.396	1.061	1.836	0.017
Grading	1.219	0.874	1.700	0.243	1.131	0.829	1.543	0.438
p53 immunostaining	1.186	0.829	1.698	0.351	1.459	1.054	2.022	0.023
TP53 FISH	0.853	0.593	1.227	0.391	1.095	0.745	1.610	0.643

FISH, fluorescence *in situ* hybridization; UICC, Union for International Cancer Control; HR, hazard ratio.

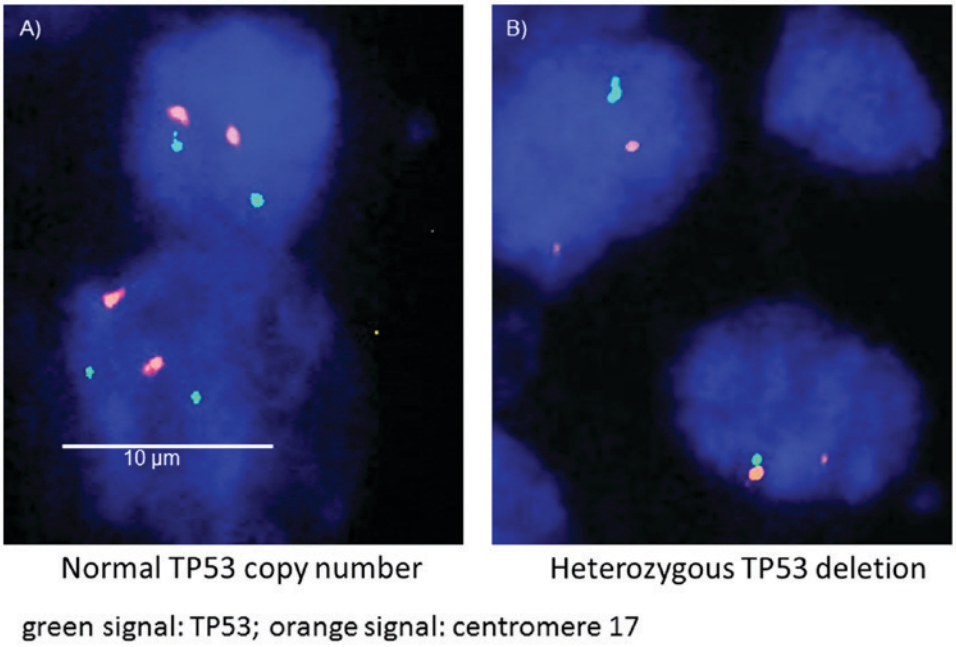

Figure 2. Representative images of fluorescence *in situ* hybridization results using the TP53 deletion probe. (A) Normal TP53 copy numbers are indicated by two green TP53 signals and two orange centromere 17 signals. (B) Heterozygous TP53 deletion is indicated by the lack of one green TP53 signal. Scale bars, 10 μm.

common gene mutation in EC with reported mutation rates between 40-70% (4,7,24). The variance of the reported mutation frequencies is in concordance to immunohistochemical confirmation studies for SSC and the results revealed from our study (19,25-28). In contrast, data from large patient cohorts concerning p53 expression in esophageal adenocarcinoma are hardly available. A recent review article by Chen and colleagues analyzed biomarkers in esophageal cancer and found only 2 studies focusing on p53 expression in esophageal adenocarcinoma with 97 patients included in the meta-analysis (14). These studies also showed p53 protein alterations in 40-65%, although the case load is too small to draw general conclusions. Our group has previously shown that the applied IHC protocols, for prostate cancer, reveal high concordance of IHC data with sequencing results, arguing for a strong validity of our IHC protocol (22).

p53 is physiologically expressed by numerous cell types and plays a major role in cell cycle control. It is considered one of the most important tumor suppressor genes (9). p53 mutations have been found in multiple cancers with large variances in the mutation frequency, depending on the observed tumor entity (29).

In our study heterozygous *TP53* deletions were found in 41% of AC and 19% of SCC. Only little data on *TP53* gene deletions have yet been reported. Recently, a study on 40 patients suffering from esophageal squamous cell carcinoma revealed heterozygous deletion of *TP53* in 22 patients

(55%). A correlation with clinical data could not be established in this study (20). The deletion rate is somewhat higher than it is in our data, which is partially caused by our stringent criteria for defining *TP53* deletions. These were applied to avoid false deletion calling due to truncation of the nuclei during tissue sectioning. Most samples with *TP53* deletion had >80% cells with fewer *TP53* fluorescence signals than centromere 17 signals. In contrast to other studies, we did not use automated systems for detection of particular gene deletions which is another possibility for deviating deletion rates compared to previous published reports (20). As already shown in prostate cancer on a TMA with >11,000 patient samples, 100% concordance between array-CGH detected deletions and FISH could be achieved for *PTEN* and *TP53* deletions using these criteria (22,23,30). Therefore, differences in the deletion rate are explained by different protocols calling more deletions as present within one patient sample. The deletion rate of 19% supports the assumption that *TP53* deletions have only minor significance as a pathway for p53 inactivation in esophageal SCC. In line with the results revealed by our study, the impact of p53 mutations is of higher importance with respect to tumor progression than the heterozygous gene deletion of chromosome 17p13. These findings also match a Northern blot analysis on expression of the p53 gene in esophageal tumorigenesis. RNA was extracted from tumor, Barrett's epithelium, and histologically normal esophageal mucosa. p53 was found to be overexpressed in cancerous or metaplastic tissue in comparison to normal tissue. Thus, the authors concluded that p53 is implicated in the progression of Barrett's epithelium to invasive cancer (31).

By analyzing p53 immunostaining and *TP53* deletions, we were able to correlate p53 IHC and *TP53* deletion status with corresponding clinical data of our patient cohort. Patients with p53 overexpression in IHC presented with shortened OS and unfavorable clinic-pathological data, meaning that p53 mutations must have an influence on tumor biology. This effect is also paralleled by a significant correlation between advanced tumor stages and high p53 expression levels in AC (Table I: Tumor stage (P=0.019), UICC stage (P=0.004), grading (P=0.027) and surgical resection margin status (P=0.006)). Furthermore, the multivariate analysis of p53 immunostaining in a setting with various established prognostic factors for esophageal cancer, such as UICC stage, distant metastasis, resection margin and grading revealed that p53 accumulation is an independent prognostic marker in SCC. In contrast, in our analysis, heterozygous *TP53* deletions do not affect the OS, neither in AC nor in SCC. These results confirm the findings of former studies in EC and other tumor entities that mono-allelic protein expression is sufficient for regular cell cycle control mediated by p53 (29). Our data show, that mono-allelic *TP53* deletion cannot be considered a major driver for tumor progression in esophageal cancer while there is no mutation in the remaining TP53 allele.

Since the poor outcome of patients with strong p53 immunostaining was independent of the TP53 deletion status, it is tempting to speculate that these cancers may at least carry dominant negative mutations with complete inactivation of wild-type p53 protein through complex formation with mutant p53 protein. This mechanism is known to lead to massive nuclear accumulation of inactive p53 complexes composed of mutated and non-mutated p53 protein (29). While IHC reveals both mutated and non-mutated p53, cells with accumulation of inactive p53 complexes are the ones that will predominantly show strong staining. Thus, strong p53 immunostaining in our samples indicates an accumulation of unfunctional p53.

Alterations of p53 have been found in virtually every region of the protein but only a handful of the most frequently occurring mutations haven been studied in depth for their contribution to cancer progression (32). In EC, the majority of patients present with *TP53* exon single nucleotide mutations (approximately 70%) while frameshift mutations are much less frequent. Only 15% of cases have been reported to have insertions or deletions as found by genome wide sequencing and PCR exon analysis (6,32). Numerous *in vitro* and *in vivo* studies confirmed the ability of mutant p53 to drive enhanced cancer invasion and motility, with evidence that mutant p53 can enhance signaling through receptors such as transforming growth factor β (TGFβ) or epidermal growth factor receptor (EGFR). Additionally, although mutated p53 has generally lost the ability to bind p53 DNA binding regions in target gene promoters, various p53 mutants can bind directly to DNA with some degree of selectivity and may thereby control the transcription of certain genes (33,34). Furthermore, there is increasing evidence that mutated p53 has an inhibitory effect on transcription factors, such as TAp63, which is of regulative importance for numerous miRNA with important roles in cancer invasion and progression (35,36).

In contrast to other cancers, such as prostate cancer, the combination of gene deletion and mutation status does not increase the malignant potential in esophageal cancer (22). In esophageal adenocarcinoma only 53 (21%) patients showed *TP53* deletion combined with strong p53 immunostaining, the rate being even lower in SCC with only 11%. These low frequencies suggest that biallelic inactivation, which is associated with a total loss of functional p53, is a catastrophic cellular event related with a high level of apoptosis. Studies using transgenic mouse models carrying heterozygous *TP53* deletions show larger and more mammalian tumors than animals with two wild type *TP53* alleles (37). Moreover, the results from animal studies confirm the findings in our study, as these results indicate that loss of both *TP53* alleles is not a prerequisite for tumor formation and that mere reduction in p53 levels may be sufficient to promote tumorigenesis.

Other studies have examined serum levels of p53 antibodies and their correlation to outcome. Shimada *et al* (38) reported on 28 patients with SCC that were positive for serum p53 antibodies out of a cohort of 105 cancers. These patients' survival was significantly worse than that of seronegative patients. To our knowledge, no correlation between serum p53 antibody levels and p53 expression in cancer tissue has yet been described for esophageal cancer. Our study does not provide any data on this issue since serum p53 antibodies were not examined in our cohort.

Seeing that cancers with strong p53 expression are correlated with poor survival independent of the established clinic-pathological prognostic markers supports the notion that these patients might profit from neoadjuvant and adjuvant therapeutic regimes in a multimodal setting. Tumor size, nodal

status and resection margin appear to not be the only relevant markers for prediction of survival in EC and therefore the search for prognostic biomarkers is warranted even in this highly malignant disease with poor overall survival even in less advanced stages.

In summary, the results of our large-scale TMA analysis in esophageal adeno- and squamous cell carcinoma show that different types of p53 alterations characterize subgroups of patients with different outcomes. Strong p53 expression is correlated with unfavorable prognosis in esophageal cancer and represents an independent prognosticator in SCC. Furthermore, homozygous *TP53* deletions are catastrophic cellular events related with a high level of apoptosis.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

NM, SN, MK, RS and CHM conceived and designed the study, and acquired, analyzed and interpreted the data. SS, AH, EB, FJ, WW, AQ, MB, KG and MT were involved in drafting the manuscript, made substantial contributions to conception, analysis and interpretation of data and revised it critically for important intellectual content. JI, GS and FG made substantial contributions to study design, acquisition of data and gave final approval of the version to be published.

Ethics approval and consent to participate

Informed consent was not required due to the retrospective nature this study. Approval for manufacturing and analyzing tissue microarrays made from the tissue samples of anonymized donors was obtained from our local review board, the Ethics Commission of the Ärztekammer Hamburg (no. WF049/09).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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