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ORIGINAL ARTICLE – CANCER RESEARCH

Fibroblast growth factor receptor 4 (FGFR4) as detected by immunohistochemistry is associated with postoperative residual disease in ovarian cancer

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Abstract

Purpose Fibroblast Growth Factor Receptor 4 (FGFR4) was proposed to hold prognostic significance in high-grade serous ovarian carcinoma (HGSOC). However, information on this deriving from large, representative patient panels is still missing, though such data would be indispensable to validate suitability of FGFR4 as prognostic marker or even pharmacological target.

Methods 1063 ovarian cancer cases were included in this study. Immunohistochemistry (IHC) was performed using two different anti-FGFR4 specific antibodies (HPA027273, sc-124) on an automated staining system. IHC data of both FGFR4 antibodies were available from 995 cases. FGFR4 immunostaining was correlated to prognostic factors including survival using uni- and multivariate proportional hazard models.

Results FGFR4 was positively associated with advanced FIGO stage, high grade and presence of residual disease. When progression free (PFS) of FGFR4 negative vs. positive patients was compared, patients scored as FGFR4 positive had significantly shortened PFS as compared to those that stained negative. All associations of FGFR4 and shortened PFS were lost during multivariate testing. No significant associations were found in terms of OS.

Conclusions We were not able to confirm FGFR4 as an independent negative prognosticator as described before. However, FGFR4 was highly prevalent in those cases harboring residual disease after debulking surgery. Since especially patients that could only be debulked sub-optimally may benefit from targeted adjuvant treatment, tyrosine kinase inhibitors targeting FGFRs might turn out to be an interesting future treatment option.

Keywords FGFR4 · Ovarian cancer · Prognosis

Background

High grade serious ovarian cancer (HGSOC) remains to be the most lethal gynaecological malignancy in developed countries (Ferlay et al. 2007; Yamagami et al. 2017). Complete surgical resection and state of the art chemotherapy have resulted in modest gains in survival, however, most HGSOC patients will ultimately die from this disease (Perren et al. 2011; du Bois et al. 2010; du Bois et al. 2009). During the course of disease HGSOC patients commonly experience multiple cancer relapses and each will be treated by chemotherapy with or without surgical debulking (Jänicke 1999). Quite often multiplication of chemotherapy associated toxicities turn to be a treatment limiting condition. In addition response rates of relapsed HGSOC are low and early progression is commonly observed (Jiang et al. 2017). Hence, there is an urgent clinical need for innovative

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targeted treatment options that may help to improve prognosis of HGSOC.

Kinase inhibitors either blocking Fibroblast Growth Factor Receptors (FGFRs) only or exerting dual blockade on FGFRs in combination with VEGFR have been demonstrated to mediate anti-tumor activity in different kinds of solid tumors (Tabernero et al. 2015; Jonker et al. 2011). FGFs as well as the FGF-receptor family have been highlighted to mediate pro-proliferative, angiogenic and anti-apoptotic signaling in ovarian cancer cells and animal models (Knights and Cook 2010). There is increasing evidence that those cancer cells carrying *FGFR* mutations or overexpressing FGF/FGFRs may undergo a FGFR mediated phenomenon termed 'oncogenic addiction' (Knights and Cook 2010; Weinstein 2002). Within this scenario—though cancer cells acquire abnormalities in several oncogenes—a certain gene product evolves to be crucial for cancer cell survival and, therefore, provides an 'Achilles heel' for neoplastic growth. Especially those 'driver' genes mediating cancer cell growth are supposed to display exceptionally interesting targets to be exploited in anti-cancer treatment (Weinstein 2002). Different kinase inhibitors have shown to convey antitumor activity to variable extents in early phase clinical trials on HGSOC (Pujade-Lauraine et al. 2016; Tolcher et al. 2015). With respect to gynecological malignancies VEGFR (targeted by Bevacizumab) and HER2 (targeted by e.g. Trastuzumab) display prominent examples that have revolutionized cancer therapy.

So far there are four FGFR genes known in humans. Though all the four (FGFR1-4) display similarities regarding molecular structure, ligand-binding properties and signaling, there are distinct features that separate FGFR4 from the other FGFRs (Heinzle et al. 2014). For instance, while FGFR1-3 undergo alternative splicing in a key extracellular ligand binding domain, FGFR4 misses this splicing site resulting in constantly high and specific ligand binding affinity of FGFR4 (Heinzle et al. 2014; Holzmann et al. 2012). This molecular feature is postulated to make FGFR4 exceptionally suited for being selectively targeted by therapeutic antibodies or antibody–drug conjugates (Heinzle et al. 2014).

Blocking FGFR4 inhibited downstream signaling of wnt, NFkB and MAPK signaling cascades (Zaid et al. 2013). siRNA mediated silencing of FGFR4 has been demonstrated to reduce growth in human ovarian cancer cells and mice (Zaid et al. 2013).

In contrast, the presence of a polymorphism (G388R, rs351855) within the *FGFR4* gene has been reported to increase platinum sensitivity in ovarian cancer cases and to predict favorable overall survival (Marme et al. 2012). However, the biological effect of G388R on protein function still remains to be largely unknown. There are two studies having investigated whether FGFR4 overexpression in ovarian cancer tissue impacts on patients' prognosis. Studying FGFR4 by immunohistochemistry in 183 (Zaid et al. 2013) and 74 (Hu and Cong 2015) patients, respectively, authors demonstrated FGFR4 overexpression to be clearly correlated with poor prognosis (Zaid et al. 2013; Hu and Cong 2015). Since data deriving from large, representative patient panels are missing so far, independent studies in large patient cohorts are indispensable thus to validate suitability of FGFR4 as prognostic markers or even pharmacological target. The current analysis utilized HGSOC tissue samples deriving from three different, population-based study cohorts from British Columbia, to address the prognostic relevance of FGFR4 as detected by immunohistochemistry (IHC).

Methods

Patients

This study utilized three different patient cohorts from British Columbia which have been described in detail before (Kalloger et al. 2011). The first group (termed OOU) consisted of 527 formalin-fixed paraffin-embedded tissue samples that had been diagnosed between 1984 and 2000. Samples were collected from more than 20 hospitals in British Columbia. Due to the retrospective character of the cohort, the long acquisition period and the different centers that were involved in sample collection no standardized fixation of the specimens was performed. Inclusion criteria was the diagnosis of ovarian cancer that had not been treated with chemotherapy before and that had been fully resected within primary cyto-reductive surgery. Due to these case selection criteria the OOU cohort contained a relatively small fraction of HGSOC.

The second cohort (termed VOA) was retrieved from the Gynecologic Tissue Bank at Vancouver General Hospital. This group consisted of 286 cases that had underwent surgery for ovarian carcinoma at Vancouver General Hospital between 2001 and 2008. Since all these samples had been gathered at one institution under the same pre-analytical conditions, this cohort represents high-quality tissue with short devitalization times and standardized fixation as well as tissue processing.

The third patient cohort (termed OOUE) consisted of 250 consecutively diagnosed ovarian carcinoma cases that had been diagnosed at five centers in Canada between 2006 and 2009. These samples had undergone standard paraffine embedding and processing.

All specimens have undergone expert pathology review, and details on patient diagnosis and outcomes have been thoroughly validated and previously published (Kalloger et al. 2011; Kobel et al. 2008). Cohorts were composed of

all histo-types of ovarian carcinoma. However, we restricted inside statistical analysis to HGSOC, endometrioid, clear cell and mucinous OC (with special focus on HGSOC) since these are the most common and hence clinically most relevant subtypes.

Immunohistochemistry

Tissue microarrays were assembled, sliced using a microtome and were processed using the Ventana Discovery XT automated system (Ventana Medical Systems, Tucson, AZ, USA) as per manufacturer's instructions. After standardized de-paraffinization, slides antigen retrieval was performed using Cell Conditioning 1 solution (Ventana). Two anti-FGFR4 primary antibodies (1:25 Sigma, HPA027273 and 1:10 Santa Cruz, sc-124) as well as appropriate positive and negative controls were applied. Primary antibodies and controls were incubated for 2 h without heat and detected with the ChromoMap DAB kit (Ventana). Two tissue cores were evaluated per patient and the higher FGFR4 staining intensity was graded absent (0), weak (1), intermediate (2) and strong (3), results were then binarized for subsequent analysis: low expression (absent, weak) versus high expression (intermediate, strong). In case of a positive signal, staining of both antibodies was uniformly distributed all over the tumor leading to a percentage of stained tumor cells near 100%. Due to this uniform staining pattern, percentage of stained cells did not influence the FGFR4 scoring.

Statistical methods

Statistics were performed using SPSS (IBM) v15. Uni- and multivariate proportional hazard models were used to correlate prognostic factors, progression free (PFS) and overall survival (OS). Chi-Square statistics were employed thus to test FGFR4 and clinico-pathological parameters for independence.

Results

Analysis of FGFR4 immunostaining across histotypes

In total 1032 of 1063 samples were eligible for IHC. IHC data of both FGFR4 antibodies were available from 995 cases and, therefore, further analysis was restricted to these 995 cases. Information on FIGO stage was available from 990 cases and about half of them ($n=495$) were stages as FIGO III or IV. The majority of patients (87.5%) was diagnosed with OC of low histologic differentiation (i.e. "high grade" in serous OC or G2, G3 in remaining epithelial subtypes). Optimal tumor debulking was achieved in 581 (58.4%) patients.

FGFR4 as stained by sc-124 was detected in 717 out of 995 (72.1%) ovarian cancer cases, while HPA027273 just stained 430 out of 995 (43.2%) cases. FGFR4 was completely absent, i.e. neither detected by sc-124 nor HPA027273, in one-fourth of the sample ($n=251$; 25.2%). Both FGFR4 antibodies showed a uniform IHC signal all over the neoplastic cells while no specific staining of tumor stroma and of intracellular spaces was observed. FGFR4 as detected by either HPA027273 or sc-124 was found to localize to both the cytoplasm and the cell membrane (Fig. 1). IHC scores of both antibodies were highly correlative ($p<0.001$).

FGFR4 positivity increased with clinical stage (sc-124: $p<0.001$; HPA027273: $p<0.001$; Table 1), was highly prevalent in the serous histotype (sc-124: $p<0.001$; HPA027273: $p<0.001$; Table 1, Supplementary Table 1)

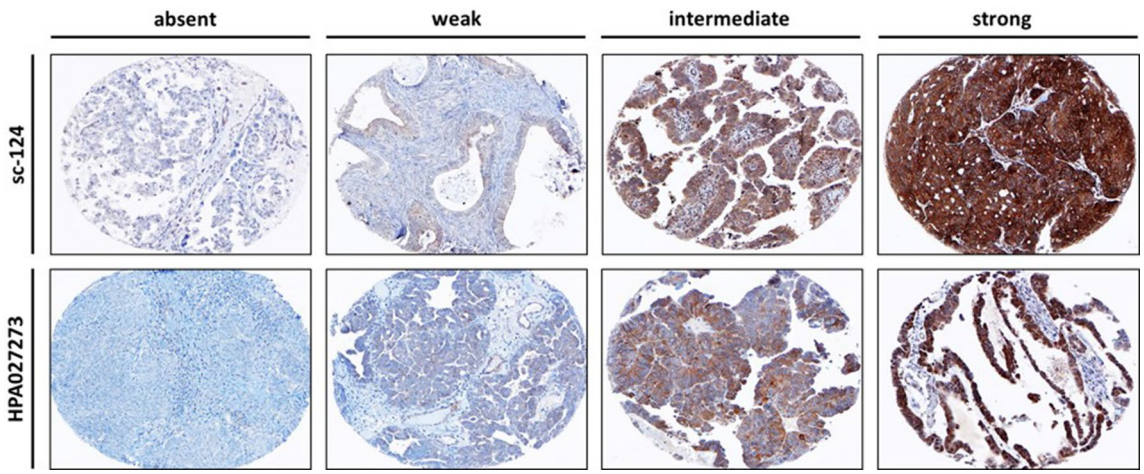

Fig. 1 FGFR4 as detected by immunohistochemistry. Representative photomicrographs of FGFR4 as stained using sc-124 (upper lane) and HPA027273 (lower lane) are presented. Photomicrographs illustrate absent, weak, intermediate and strong FGFR4 staining

Table 1 Patients' characteristics (whole sample) as correlated to FGFR4 staining

	sc-124					HPA027273				
	Negative	(%)	Positive	(%)	*p*	Negative	(%)	Positive	(%)	*p*
FIGO										
I, II	165	(33.3)	330	(66.7)		325	(65.7)	170	(34.3)	
III, IV	113	(22.8)	382	(77.2)	< 0.001	238	(48.1)	257	(51.9)	< 0.001
Grade										
G1	55	(44.4)	69	(55.6)		89	(71.8)	35	(28.2)	
G2, G3	223	(25.6)	647	(74.4)	< 0.001	476	(54.7)	394	(45.3)	< 0.001
Histotype										
Other	153	(41.9)	212	(58.1)		235	(64.4)	130	(35.6)	
HGSOC	125	(19.8)	505	(80.2)	< 0.001	330	(52.4)	300	(47.6)	< 0.001
Residual disease										
None	198	(34.1)	383	(65.9)		392	(67.5)	189	(32.5)	
Any	80	(19.3)	334	(80.7)	< 0.001	173	(41.8)	241	(58.2)	< 0.001
Age										
≤ 58 years	130	(26.3)	365	(73.7)		273	(55.2)	222	(44.8)	
> 58 years	148	(29.6)	352	(70.4)	0.241	292	(58.4)	208	(41.6)	0.301

Histologic subtype, grade, FIGO stage and residual disease after surgery are displayed. The current study reports data on those 995 cases with staining data of both antibodies (sc-124 and HPA027273) available

and was positively associated with residual disease (sc-124: $p < 0.001$; HPA027273: $p < 0.001$; Table 1) and grade (sc-124: $p < 0.001$; HPA027273: $p < 0.001$; Table 1).

The whole cohort was split up into FGFR4 negative vs. positive cases and OS as well as PFS were compared between groups. Survival analysis across histotypes revealed an association of FGFR4 with shortened PFS (sc-124: $p < 0.001$; HPA027273: $p = 0.012$; Fig. 2) regardless the FGFR4 antibody used, respectively. In the case of OS FGFR4 as stained by HPA027273 kept to predict shortened OS, though this association was of borderline significance only ($p = 0.063$).

Given considerable data suggesting etiological difference between histotypes and our observations of histotype-specific expression trends, we restricted our further analysis to the most common histotypes, HGSOC ($n = 630$), endometrioid OC ($n = 148$), mucinous ($n = 43$) and clear cell OC ($n = 148$).

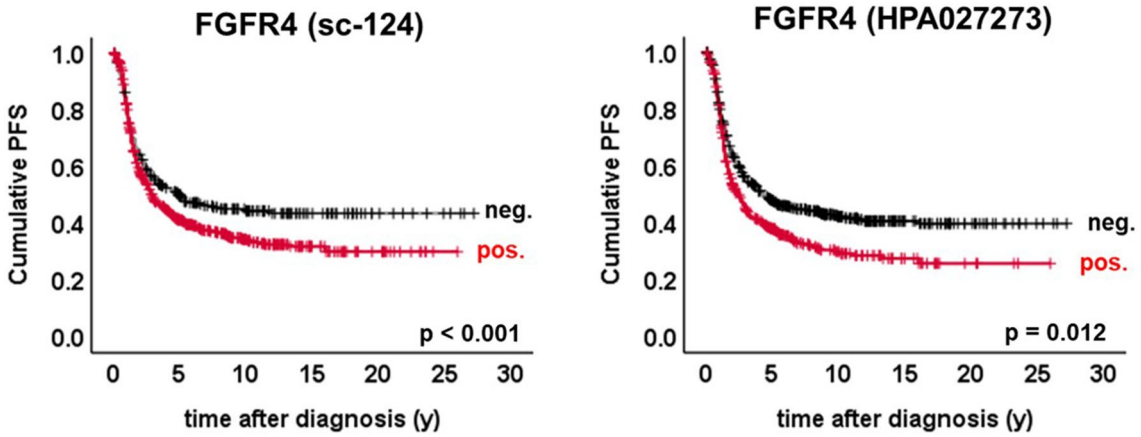

Fig. 2 Kaplan–Meier analysis for progression free survival (PFS) of FGFR4 positive vs. negative patients of the overall study cohort. Survival curves of FGFR4 negative vs. positive patients are shown. With respect to the overall study cohort (i.e. including all histological subtypes) PFS of patients scored as FGFR4 positive was significantly shorter than PFS of those patients that stained negative. This observation was true for either of the two antibodies used to detect FGFR4 (sc-124 in **a** and HPA027273 in **b**). For each survival plot a corresponding log-rank *p* value is shown

FGFR4 as correlated to clinico-pathological parameters in high grade serous, endometrioid, mucinous and clear cell OC

Most of the HGSOC patients studied were diagnosed an advanced FIGO stage (440/626, 70.3%). Advanced FIGO stage was less common in the other histotypes i.e. endometroid, mucinous and clear cell. All patients underwent surgery thus to either attempt maximal tumor debulking or—given an a priori non-curable situation—for histological confirmation of OC. Optimal tumor debulking was achieved in 254 (40.3%) HGSOC cases. Regarding the remaining histotypes, debulking rates were at least twice as high as in HGSOC (Table 2). Patient age was differentially distributed among subtypes, too (Table 2).

FGFR4 was detected in the large majority of HGSOC in case IHC staining was performed using sc-124 (505/630, 80.8%). Detection rates were significantly lower for the HPA027273 antibody (300/630; 47.6%, $p < 0.001$) when compared to the sc-124 antibody regarding both HGSOC and remaining histotypes. Though absolute positive rates were different, association of both stainings split up by histological subtype was still highly significant (HGSOC: $p < 0.001$; endometrioid: $p < 0.001$; mucinous: $p = 0.002$; clear cell: $p < 0.001$).

HPA027273 positivity appeared to rise in parallel with FIGO stage ($p < 0.001$) in HGSOC only while no such association was found in case of sc-124 or regarding the other histotypes. FGFR4 staining, as detected by HPA027273, was positively correlated to presence of residual disease in case of high grade serous ($p < 0.001$), endometrioid ($p = 0.005$) and clear cell ($p = 0.002$) OC. FGFR4 positivity rate seemed to rise in parallel with tumor grade in endometroid OC and was negatively associated with patient age in non-serous histological subtypes (Table 2). Finally, a binary logistic regression was run to assess the effects of FIGO stage, patient age and FGFR4 positivity (as stained by HPA027273) on the achievement of complete tumor resection in HGSOC. Tumors of cases diagnosed for advanced FIGO stage (OR = 43.32, 95% CI 24.53–76.52; $p < 0.001$) or FGFR4 positivity (OR = 2.85, 95% CI 1.81–4.49; $p < 0.001$) were less likely to be resected completely.

Progression free and overall survival

Positive immunostaining of FGFR4 was tested for its prognostic value with respect to OC histotypes. FGFR4 kept to be associated to shortened PFS in the HGSOC subtype only in case it was detected by the antibody HPA027273 ($p = 0.006$; Fig. 3). The association of FGFR4 and shortened PFS—which had been observed in the non-stratified cohort—was lost when HGSOC cases were stained using sc-124. In addition, FGFR4 was not correlated to PFS in endometrioid, mucinous or clear cell OC, regardless the antibody used (Fig. 3). To test whether the association HPA027273 staining is biased by debulking status, survival analysis was repeated in HGSOC patients that had been optimally and sub-optimally debulked separately (Fig. 4). Upon stratification by debulking status, prognostic significance of HPA027273 was lost. Surprisingly, we found FGFR4 as stained by sc-124 to be correlated to favorable PFS ($p = 0.047$) in patients that had been debulked sub-optimally. This association was of borderline significant only. Within multivariate testing, both associations were lost and only FIGO stage and residual disease kept to be prognostic for PFS (Table 3).

We further studied whether FGFR4 might be prognostic for OS. Using the same parameters as described in case of PFS, we did not detect any significant association of FGFR and OS, neither in the patient cohort sub-divided by histotype nor by debulking status.

Discussion

So far FGFR4 has been mainly described to comprise proto-oncogenic actions such as tumour cell invasion, proliferation, resistance to chemotherapy or blockade of apoptosis in various cancer entities (Cho et al. 2016; Li et al. 2016). In line with this, it was reported that the overexpression of FGF-1, an activating ligand of FGFR4, was prognostic for shortened overall survival in HGSOC (Birrer et al. 2007). However, there are only few reports on FGFR4 in ovarian cancer so far. First, the chromosomal region encoding FGFR4 was found to be amplified in about 25% of HGSOC cases studied (Wei et al. 2013, Birrer et al. 2007). However, it was not possible to reproduce these findings on the TCGA cohort (Cancer Genome Atlas Research Network 2011), as discussed by Zaid et al. (Zaid et al. 2013). Another retrospective analysis by our group found FGFR4 polymorphism Gly388Arg to predict prolonged overall survival and to be correlated to platinum sensitivity (Marme et al. 2012). Whether this polymorphism may confer activating or blocking effects on FGFR4, or whether it may influence protein function at all remains unknown. Zaid et al. highlighted FGFR4 immunopositivity as detected by sc-124 to reduce median overall survival from 28 to 55 months in 183 HGSOC cases studied (Zaid et al. 2013). Further, they provided in vitro and in vivo evidence, that FGFR4 may act as an oncogene (Zaid et al. 2013). Zaid et al —like us—found sc-124 to show a uniform staining throughout the tumor tissue and we both used a similar IHC scoring technique to quantify FGFR4 expression (Zaid et al. 2013). In accordance with what is known from the literature, we found FGFR4 to predict shortened PFS in the whole, non-stratified cohort. This result was confirmed using an independent, second antibody (called HPA027273)

Table 2 Patients' characteristics (sample split up into histological subtypes) as correlated to FGFR4 staining

Subtype	Antibody used to detect FGFR4	Staining result	Total	FIGO					Grade					Residual disease					Age				
				I, II	(%)	III, IV	(%)		1	(%)	2, 3	(%)		None	(%)	Any	(%)		≤ 58 y	(%)	> 58 y	(%)	
HGSOC	sc-124	Neg.	125	35	(28.0)	90	(72.0)		3	(0.9)	327	(99.1)		57	(45.6)	68	(54.4)		51	(40.8)	74	(59.2)	
		Pos.	505	151	(30.1)	350	(69.9)	0.640	1	(0.3)	298	(99.7)	0.130	197	(39.0)	308	(61.0)	0.179	217	(43.0)	288	(57.0)	0.660
	HPA027273	Neg.	330	121	(36.9)	207	(63.1)		2	(1.6)	123	(98.4)		171	(51.8)	159	(48.2)		141	(42.7)	189	(57.3)	
		Pos.	300	65	(21.8)	233	(78.2)	< 0.001	2	(0.4)	502	(99.6)	0.365	83	(27.7)	217	(72.3)	< 0.001	127	(42.3)	173	(57.7)	0.920
Endometrioid	sc-124	Neg.	63	58	(92.1)	5	(7.9)		65	(66.3)	33	(33.7)		63	(100.0)	0	(0.0)		31	(49.2)	32	(50.8)	
		Pos.	85	80	(94.1)	5	(5.9)	0.623	21	(42.0)	29	(58.0)	0.069	81	(95.3)	4	(4.7)	0.081	61	(71.8)	24	(28.2)	0.005
	HPA027273	Neg.	98	91	(92.9)	7	(7.1)		42	(66.7)	21	(33.3)		98	(100.0)	0	(0.0)		58	(59.2)	40	(40.8)	
		Pos.	50	47	(94.0)	3	(6.0)	0.793	44	(51.8)	41	(48.2)	0.005	46	(92.0)	4	(8.0)	0.005	34	(68.0)	16	(32.0)	0.296
Mucinous	sc-124	Neg.	11	9	(81.8)	2	(18.2)		12	(46.2)	14	(53.8)		8	(72.7)	3	(27.3)		5	(45.5)	6	(54.5)	
		Pos.	32	29	(90.6)	3	(9.4)	0.432	6	(35.3)	11	(64.7)	0.323	31	(96.9)	1	(3.1)	0.017	19	(59.4)	13	(40.6)	0.423
	HPA027273	Neg.	26	22	(84.6)	4	(15.4)		6	(54.5)	5	(45.5)		22	(84.6)	4	(15.4)		11	(42.3)	15	(57.7)	
		Pos.	17	16	(94.1)	1	(5.9)	0.342	12	(37.5)	20	(62.5)	0.480	17	(100.0)	0	(0.0)	0.089	13	(76.5)	4	(23.5)	0.027
Clear cell	sc-124	Neg.	75	62	(82.7)	13	(17.3)		1	(1.0)	97	(99.0)		68	(90.7)	7	(9.3)		41	(54.7)	34	(45.3)	
		Pos.	73	61	(84.7)	11	(15.3)	0.736	0	(0.0)	50	(100.0)	0.322	62	(84.9)	11	(15.1)	0.286	56	(76.7)	17	(23.3)	0.005
	HPA027273	Neg.	98	85	(86.7)	13	(13.3)		1	(1.3)	74	(98.7)		92	(93.9)	6	(6.1)		58	(59.2)	40	(40.8)	
		Pos.	50	38	(77.6)	11	(22.4)	0.156	0	(0.0)	73	(100.0)	0.474	38	(76.0)	12	(24.0)	0.002	39	(78.0)	11	(22.0)	0.023

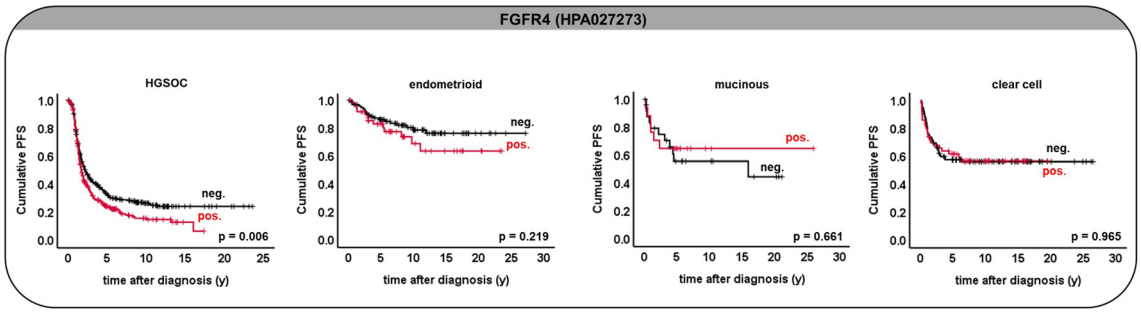

Fig. 3 Kaplan–Meier analysis for the progression free survival (PFS) of FGFR4 positive vs. negative patients as split up by histological subtype. Survival curves of FGFR4 negative vs. positive patients were computed by the Kaplan–Meier method. PFS between groups (split up by histotype **a** HGSOC, **b** endometroid, **c** mucinous and **d** clear cell) were compared by applying the log rank test. A statistically significant result was only obtained in case of HGSOC and only if the HPA027273 antibody was used to detect FGFR4. Corresponding log-rank *p* values are shown

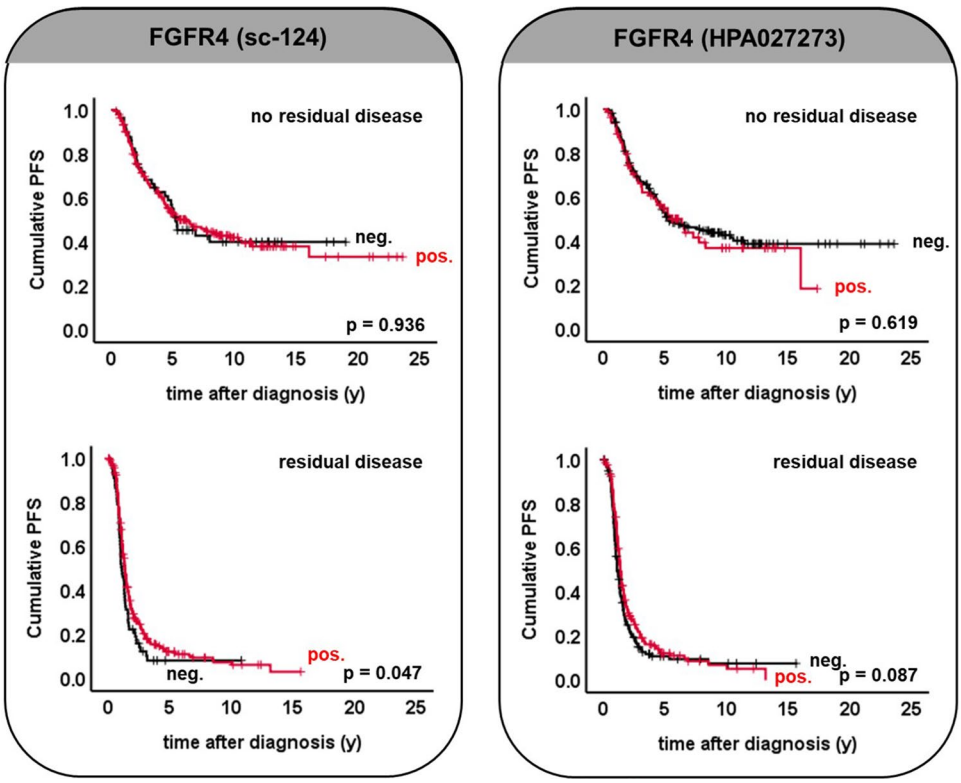

Fig. 4 Kaplan–Meier analysis for the progression free survival (PFS) of FGFR4 positive vs. negative HGSOC patients as split up by postoperative residual disease. The Kaplan–Meier method was used to build survival curves of FGFR4 negative vs. positive HGSOC patients. The study cohort was split up by residual disease status. Survival curves were compared by applying the log rank test. Only if FGFR4 was detected by sc-124 and only in patients harboring residual disease we found a weak correlation of FGFR4 and favorable PFS. Corresponding log-rank *p* values are shown

that detected a slightly different epitope on FGFR4. Staining of both antibodies was further correlated to advanced FIGO stage, high grade, serous histologic differentiation and presence of residual disease. As the aforementioned clinical co-variables might bias a potential, presumed FGFR4 dependent influence on prognosis, we repeated prior analysis after stratification of the patient cohort. The association of FGFR4—as detected by sc-124—and PFS was lost after the cohort had been split up into histotypes. Using the HPA027273 antibody the association described above still kept to be significant in the high-grade serous subtype when tested in a univariate manner. Again, significance was lost within multivariate testing. In an overall appraisal, FGFR4 expression may indicate shortened PFS in certain subgroups of OC. However, prognostic significance of FGFR4 seems to be strongly influenced by clinical and pathological covariables, e.g. residual disease as described below. Thus, conclusions on a potential prognostic value of FGFR4 need to be drawn with caution.

Potential caveats of our study include the lack of an orthogonal molecular control, such as proteomic or mRNA expression data. Both antibodies are rabbit polyclonal IgG with unique but overlapping peptide regions used as antigens. Production lot (animals) as well as the target antigens

Table 3 Multivariate Cox Regression analysis for PFS

	Exp(B)	Sig.	95.0% CI for Exp (B)	
			Lower	Upper
FIGO I, II vs. III, IV	1.794	< 0.001	1.325	2.429
Residual disease (none vs. any)	2.711	< 0.001	2.059	3.569
Age (≤ 58 years vs. > 58 years)	0.940	0.514	0.780	1.133
FGFR4 (sc-124; Score 0, 1 vs. 2, 3)	0.850	0.171	0.674	1.072
	Exp(B)	Sig.	95.0% CI for Exp (B)	
			Lower	Upper
FIGO I, II vs. III, IV	1.821	< 0.001	1.347	2.463
Residual disease (none vs. any)	2.739	< 0.001	2.073	3.618
Age (≤ 58 years vs. > 58 years)	0.940	0.515	0.780	1.133
FGFR4 (HPA027273; Score 0, 1 vs. 2, 3)	0.886	0.217	0.731	1.074

may dictate differences in each antibody's specificity, on/off-target range of epitope recognition, and binding affinity—all of which will affect the interpretable signal from IHC. Further, though both staining methods had been thoroughly validated by positive as well as negative controls, unspecific staining or cross reactivity with other proteins may not be fully excluded. Ultimately, highly specific, reproducible, protein or mRNA-based detection will be indispensable to understand any prognostic or biological value of FGFR4 in HGSOC.

Despite the lack of a robust, independent association with survival, FGFR4 may still have biological value in the regulation of disease progression or etiology of HGSOC. In particular, our results suggest FGFR4 expression to be more common in HGSOC cases that are not fully resected. It may be hypothesized that this association may be due to FGFR4 mediated tumor aggressiveness—and thus may support the observations made by Zaid et al. (Zaid et al. 2013). Though this may seem a logical conclusion, a statement on this needs to be interpreted with care, since information on why these patients were not completely resected was missing. In general, this is not the first-time biological signals have been tied to tumor resectability. Riester et al. performed extensive analysis to identify a mRNA signature associated with sub-optimal debulking (Riester et al. 2014). FGFR4 was detected in up to 80.7% of sub-optimally debulked patients. As nowadays residual disease is supposed to be the most important predictor for shortened PFS, sub-optimally debulked women may benefit from additional, optimized adjuvant oncologic treatment. Hence, targeted treatment approaches accompanying standard platinum-taxane-based chemotherapy are highly warranted. Whether tyrosine kinase inhibitors targeting FGFRs and VEGFR, such as Lenvatinib, may be effective in women harboring macroscopic residual disease after debulking surgery, may be an interesting question to be addressed by a clinical trial.

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Availability of data and material The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest Sabine Heublein received research support from Novartis, Ferring, Apceth and Addex; Honoria from Roche, travel expenses from Astra Zeneca. Frederik Marmé received honoraria from Roche, AstraZeneca, Pfizer, Clovis, Tesaro, Amgen, Celgene, Eisai, MSD, Genomic Health, Curevac; Travel expenses from Roche, Pfizer, Astra Zeneca, Pharma-Mar, Amgen, Celgene; Advisory Boards for Roche, Astra Zeneca, Pfizer, MSD, Amgen, Celgene, Genomic Health, Curevac. Stefan Kommoss and Michael S. Anglesio declare no competing interests.

Ethics approval Collection of patient specimens and data from the British Columbia cohort was done under approved research protocols reviewed by the British Columbia Cancer Agency and University of British Columbia research ethics board (H05-60119).

Informed consent No written informed consent of the participants is needed given the circumstances described above.

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