Sulfatase-2: a prognostic biomarker and candidate therapeutic target in patients with pancreatic ductal adenocarcinoma

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Background: Pancreatic ductal adenocarcinoma (PDAC) is the fifth most common cause of cancer death in the UK. Its poor prognosis is attributed to late detection and limited therapeutic options. Expression of SULF2, an endosulfatase that modulates heparan sulfate proteoglycan 6-O-sulfation and is reportedly tumourigenic in different types of cancer, was investigated.

Methods: SULF2 expression was determined immunohistochemically in archival surgical resection tissue sections from 93 patients with a confirmed histological diagnosis of PDAC between 2002 and 2008 followed for a median of 9 years. Relationships with clinico-pathological parameters and patient survival were explored.

Results: The majority of PDACs showed positive SULF2 staining in tumour cells and intratumoural or tumour-adjacent stroma. Greater than 25% SULF2-positive tumour cells was present in 60% of cancers and correlated with tumour stage (P = 0.002) and perineural invasion (P = 0.024). SULF2 intensity was scored moderate or strong in 81% of cancers and positively correlated with vascular invasion (P = 0.015). High SULF2 expression, defined as >50% SULF2-positive tumour cells and strong SULF2 staining, was associated with shorter time to radiological progression (P = 0.018, HR 1.98, CI 1.13–3.47). Similarly, by multivariate analysis, high SULF2 expression was independently associated with poorer survival (P = 0.004, HR 2.10, CI 1.26–3.54), with a median survival of 11 months vs 21 months for lower PDAC SULF2.

Conclusions: Elevated SULF2 in PDAC was associated with advanced tumour stage, vascular invasion, shorter interval to radiological progression and shorter overall survival. SULF2 may have roles as a prognostic biomarker and as a therapeutic target for patients with PDAC.

Each year, ~8800 people are diagnosed with pancreatic ductal adenocarcinoma (PDAC) in the UK (Cancer Research UK (Cancer Statistics)). A significant proportion of cancer-related death is attributed to PDAC (Siegel et al, 2012) with a 5-year survival rate in the region of 6% (DeSantis et al, 2014). PDAC is asymptomatic in early stages and its poor prognosis is attributed to late detection,
as well as the aggressive nature of the disease (Chrysoyta et al., 2013). Consequently, only 10–15% of those affected are candidates for surgical resection, with a 5-year survival post resection of just 10–25% owing to frequent regional invasion and distant micrometastases at the time of resection (Heinemann and Boeck, 2008). The current palliative chemotherapy regimens for those with later stage disease include gemcitabine with or without nab-paclitaxel, or FOLFIRINOX and provide modest benefit with a median overall survival of <1 year (Conroy et al., 2011; Thota et al., 2014).

Clinically useful biomarkers which inform prognosis or aid treatment stratification for those with PDAC are presently lacking. The contribution of extracellular microenvironment (ECM) to the development and progression of PDAC is recognised (Apte et al., 2015), as is a role for the pro-angiogenic vascular endothelial growth factor (VEGF), which is expressed in >90% of cases, and associated with both liver metastases and shorter survival of patients (See et al., 2000). ECM interactions with angiogenic as well as proliferative signalling pathways are closely regulated by heparan sulfate proteoglycans (HSPGs). VEGF is one of the HSPG-bound proteins, which include other ligands relevant to PDAC progression such as FGF-1 and FGF-2, Wnts and ECM components such as collagens, fibronectin and laminin which are present in PDAC stroma. The heparan sulfate (HS) chains of HSPGs are rich in uronic acid moieties and sulfate groups that are negatively charged, producing binding sites for growth factors, receptors and ECM molecules. Charge, and therefore sequestration vs release of growth factors, as well as orientation and binding affinity, are regulated by the degree of sulfation – particularly at the 6-O position of HS chains (Pye et al., 1998, 2000; Ai et al., 2003). 6-O-Sulfation is in turn regulated by sulfotransferases which add sulfate groups and the extracellular endosulfatases, sulfatase-1 (SULF1) and sulfatase-2 (SULF2), which remove them (Morimoto-Tomita et al., 2002; Saad et al., 2005; Staples et al., 2011).

SULF2 has been reported to be upregulated and to have a candidate oncogenic role in a number of cancers, including glioblastoma, liver, lung, gastric, oesophageal and prostate cancers (Hur et al., 2012; Lai et al., 2008, 2010; Lemjabbar-Alaoui et al., 2010; Lui et al., 2012; Phillips et al., 2012). In PDAC, Nawroth et al. (2007) have previously reported an increase in SULF2 mRNA in cell lines and increased SULF2 protein expression relative to non-tumour tissues expression in a small number of cases. shRNA-mediated SULF2 knockdown or transfection with a catalytically inactive dominant form of SULF2 enzyme in 3 PDAC cell lines reduced cell growth in vitro and tumourigenicity in vivo (Nawroth et al., 2007). In this study, we have assessed SULF2 as a candidate immunohistochemical prognostic biomarker in a cohort of 93 patients who underwent PDAC resection. In the majority of cases SULF2 was overexpressed in PDAC cells, with the percentage of positive cells correlating with tumour stage, and the SULF2 intensity (SI) correlating with vascular invasion. Combining percentage of positive cells and SI, high-PDAC SULF2 was associated with vascular invasion, a shorter time to radiological progression and poorer overall survival.

MATERIALS AND METHODS

Patient cohort. As part of a pilot study, SULF2 expression was assessed in formalin-fixed paraffin-embedded tissues (FFPE) from 19 patients undergoing resection for PDAC and consenting to the use of their tissues for research projects, governed by the Newcastle upon Tyne Hepatopancreatobiliary Research Tissue bank. The data presented include these cases, in combination with data generated subsequently from a larger retrospective series (an additional 74 cases), exploring SULF2 expression in archived cases from patients with a confirmed histological diagnosis PDAC. Study of the retrospective case series was approved by the Newcastle and North Tyneside Regional ethics committee (REC approval 11/H10908/02) on 03 March 2011. The selection interval was from 2002 to 2008 and the REC waived the need for informed consent. Patient confidentiality was respected at all times and analyses were on code-linked anonymised data sets. The Newcastle upon Tyne NHS Foundation Trust Research and Development department approved this project.

The study is classed as a retrospective case series, including patients undergoing resection with curative intent, in whom a diagnosis of PDAC was histologically confirmed. Additional inclusion criteria included the availability of FFPE blocks for study. Patients with known distant metastases at the time of resection were excluded, as were those with histologically benign disease or cholangiocarcinoma.

Clinico-pathological information, including Union for Internation Cancer Control tumour node metastases (TNM) stage, tumour grade, vascular, lymphatic and perineural invasion, lymph node status, resection margin status, time to progression (TTP) and patient survival, was collected from histopathology reports, radiology reports and patient records. Patient demographics and clinico-pathological data are shown in Table 1. The patients were followed-up for a median of 9.4 (range 6.1 to 12.9) years until 31 December 2014.

Immunohistochemistry. SULF2 immunohistochemistry was performed on a Benchmark Ultra autostainer (Ventana, Tucson, AZ, USA) using anti-SULF2 antibody (catalogue number MCA5692GA, AbD Serotec, Oxford, UK) at a dilution of 1:150. In brief, the tissue sections were incubated in primary antibody for 32 min following heat-induced epitope retrieval using CC1 buffer (Ventana) for 6 min at 100 °C. Detection and visualisation was achieved using an OptiView IHC DAB Detection Kit and 4 min of OptiView Amplification (Ventana). Control cases without primary antibody confirmed an absence of non-specific staining. Examples are included in Supplementary Figure 1.

Scoring method. An expert pancreatic pathologist (BH) blinded to patient outcome assessed the SULF2-immunostained slides at ×100 magnification. The percentage of SULF2-positive carcinoma cells was semi-quantitatively assessed with a score from 0–4 (0 = no carcinoma cells positive, 1 = 1–25% carcinoma cells positive, 2 = 26–50% carcinoma cells positive, 3 = 51–75% carcinoma cells positive and 4 = 76–100% carcinoma cells positive). The SULF2-specific SI was assessed with a score from 0–3 (0 = no staining, 1 = weak, 2 = moderate, 3 = strong). Representative cases for each score are shown in Figure 1. If a range of SI was noted the predominant score was used. The semi-quantitative score of SULF2-positive PDAC cells and the intensity score were added and a summative combined score (range 0–7) was created for each case. In addition, the extent of stromal staining within the tumour and outside the tumour was scored from 0 to 2 (0 = no staining, 1 = focal staining, 2 = extensive staining). Staining of benign and dysplastic epithelium or of tissues unrelated to the tumour was recorded. Presence of endothelial staining was recorded on all cases as an internal positive control. A second experienced pathologist (DT) also blinded to patient outcome, evaluated 10 random SULF2-immunostained slides (~10% of cases). Inter-observer agreement assessed by the Kappa measure was very good for percentage of SULF2-positive tumour cells (0.75) and perfect (kappa statistic 1) for SULF2 SI.

Statistical analysis. All statistical analyses were conducted using SPSS statistical package (IBM, version 22). Percentage of SULF2-positive cells and SI were treated as ordinal categorical variables. To identify relationships between percentage of SULF2-positive cells or SI with clinico-pathological features, cross-tabulation and χ²-tests were performed. The Monte Carlo correction was applied.
RESULTS

Demographic data for the case series studied. Details of the case series of 93 patients undergoing elective curative surgery for PDAC are summarised in Table 1. The median age was 65 years and 53 were male. The vast majority (92/93) underwent a Whipples’ pancreatectoduodenectomy for tumours in the head or neck of the pancreas. In one case the tumour was in the body and a total pancreatectomy was performed. None of the patients received preoperative chemotherapy. The series did not include patients with known metastatic disease and 70/93 were classed as having TNM stage 3 disease. Similarly, 61/93 tumours were graded as poorly differentiated, with the majority having vascular, perineural or lymphatic invasion (Table 1). Forty-six patients (50%) received adjuvant chemotherapy. The commonest adjuvant regime (36 patients) was fluorouracil (5FU) and folinic acid, either as the standard of care or as part of the ESPAC III trial. Similarly, six patients received adjuvant gemcitabine. One patient received the DNA methyl transferase inhibitor MG98, another received capcitabine, whereas in two patients, the adjuvant regime was not documented. Thirty two of 88 patients were documented as receiving palliative second-line chemotherapy, most often gemcitabine, whereas in two patients, the adjuvant regime was not documented. Thirty two of 88 patients were documented as receiving palliative second-line chemotherapy, most often gemcitabine, whereas two patients entered clinical trials. As a number of patients follow-up palliative care was outside of our tertiary referral centre, TTP was recorded as first documented ‘radiological’ progression. Consequently the data set was skewed towards those patients fit enough to undergo active monitoring, with analyses on progression being limited to the 67 patients in whom this clearly defined end point was available. Survival data were available in all 93 patients.

Elevated SULF2 expression was common in PDAC and the percentage of cells positive correlated with tumour stage. SULF2 was consistently identified in endothelial cells and relatively weak expression also observed in benign atrophic ductal epithelium. Weak-to-moderately intense cytoplasmic staining was occasionally observed in dysplastic epithelial cells of PanINs, in islet and acinar cells, periductal glands and benign ductal epithelial cells. Examples of these are shown in Supplementary Figure 1, as is variable staining that was present in lymph node sinuses, adipocytes and inflammatory cells. In contrast, expression of SULF2 in PDAC was frequently of greater intensity (Figure 1). In the PDAC cells, SULF2 was observed in the cytoplasm with a granular quality. Focal membranous staining was identified in tumours with clear cell morphology. Sixty per cent (56 out of 93) of PDACs contained >25% of cells positively stained for SULF2. A summary of the immunohistochemical results is shown in Table 1.

The percentage of SULF2-positive tumour cells correlated significantly with tumour stage (P = 0.002), the majority (16 out of 22; 73%) of cases with >75% tumour cells positive being stage T3, with local invasion. The percentage of SULF2-positive PDAC cells also correlated with perineural invasion (P = 0.024).

Cytoplasmic SULF2 expression in intratumoural stromal fibroblasts was either focal or marked in 69% (64 out of 93) of PDAC. SULF2 was also evaluated in the stroma outside the tumours and similarly showed either focal or marked staining in the majority of cases (76 out of 93; 82%). Stromal expression of SULF2 did not correlate with either PDAC grade or stage. SULF2 intensity in PDAC was positively associated with the presence of vascular invasion. In all, 81% (75 out of 93) of cancers showed moderate or strong intensity of SULF2 expression.
The intensity correlated significantly with the presence of vascular invasion ($P = 0.015$).

The SULF2 combined score correlated with the presence of vascular invasion. The combined SULF2 IHC score of percentage cells positive and the level of intensity, as shown in Table 1, correlated significantly with the presence of vascular invasion ($P = 0.026$, Pearson’s $\chi^2$, linear by linear association).

High-PDAC SULF2 was associated with a shorter time to radiological progression. Radiological TTP was documented in 67 out of 93 (62%), with the median TTP being 11.6 months. One factor associated with radiological TTP was lymph node positivity ($P = 0.007$, HR 4.15, CI 1.49–11.58). Age, sex, tumour grade, tumour stage, resection margin status, vascular invasion, lymphatic invasion and perineural invasion had no significant impact on TTP in this relatively small series. Considering the SULF2 immunohistochemistry (IHC) scores applied, both ‘> 75% PDAC cells SULF2 positive’ or SULF2 SI scored as ‘strong’, were associated with significantly shorter TTP. Combining the IHC scores, as shown in Table 1, identified those cases with a combined score of 6 or 7 as having a poorer outcome. The median TTP was 8 months in this combined group, vs 13 months in combined groups scoring 0–5, as shown in Supplementary Figure 2 ($P = 0.018$, HR 1.98, CI 1.13–3.47). Both lymph node metastasis and the combined SULF2 score (groups 0–5 vs 6–7), were independently associated with a shorter TTP (lymph nodes positive $P = 0.006$, HR 4.28, CI 1.53–12; high SULF2 $P = 0.014$, HR 2.05, CI 1.16–3.63, multivariate Cox regression; Supplementary Table 1).

Of the 67 cases with documented radiological TTP, 36 received adjuvant chemotherapy. Adjuvant chemotherapy had no significant impact on radiological TTP. Standard histopathological

Figure 1. SULF2 expression in pancreatic ductal adenocarcinoma. Representative immunohistochemical images of PDAC cases showing different percentages of SULF2-positive tumour cells (0–100%) and SI of SULF2 in tumour cells. SI 0: none, SI 1: weak, SI 2: moderate, SI 3: strong staining. (A) shows endothelial cell SULF2-positivity used as internal positive control. CS = combined SULF2 score.
High-PDAC SULF2 was significantly associated with shorter overall survival. The overall mortality during the period of follow-up was 94% (87 out of 93), with a median of 16.5 months post resection until death (range 0.3–148, s.d. 30). Factors significantly associated with survival post resection included age, lymph node metastasis and receipt of adjuvant chemotherapy, as assessed by univariate Cox regression analysis (Table 2). Regarding SULF2, those cases with >75% PDAC cells positive had a poorer outcome, as did those cases with strong SI compared with cases without SULF2 expression (Table 2). PDAC with a combined SULF2 score 6 or 7 had a significantly poorer overall survival (Table 2). Comparing combined groups 0–5 vs 6–7, the median

### Table 2. Factors associated with survival

| Variable                              | n   | Univariate analysis |                      | multivariate analysis |                      |
|---------------------------------------|-----|---------------------|----------------------|-----------------------|---------------------|
|                                       |     | P value             | HR                   | 95% CI                | P value             | HR                   | 95% CI                |
| Age (median 65.3 years)               | 93  | 0.031*              | 1.03                 | 1.00–1.05             | 0.462               | 1.01                 | 0.99–1.04             |
| Sex (male/female)                     | 53/40 | 0.573               | 1.13                 | 0.74–1.73             | Not included        |                      |                      |
| PDAC TNM stage                        |     |                     |                      |                       |                     |                      |                      |
| 1 = Within pancreas < 2 cm            | 3   | 0.595               | 2.17                 | 0.50–9.38             | Not included        |                     |                      |
| 2 = Within pancreas > 2 cm            | 19  | 0.299               | 2.44                 | 0.59–9.99             |                      |                     |                      |
| 3 = Local invasion                    | 70  | 0.217               | 3.92                 | 0.35–43.9             |                      |                     |                      |
| 4 = Distant spread                    | 1   | 0.268               | 3.92                 | 0.35–43.9             |                      |                     |                      |
| PDAC grade                            | 31  | 0.676               | 2.44                 | 0.59–9.99             |                      |                     |                      |
| Poor                                  | 61  | 0.443               | 2.17                 | 0.30–15.8             |                      |                     |                      |
| Resection margin (R0/R1)              | 28/65 | 0.247               | 1.32                 | 0.83–2.10             | Not included        |                     |                      |
| Vascular invasion (no/yes)            | 16/77 | 0.170               | 1.49                 | 0.84–2.65             | Not included        |                     |                      |
| Perineural invasion (no/yes)          | 2/91 | 0.128               | 4.66                 | 0.65–33.6             | Not included        |                     |                      |
| Lymphatic invasion (no/yes)           | 33/60 | 0.208               | 1.33                 | 0.85–2.09             | Not included        |                     |                      |
| Lymph nodes +ve (no/yes)              | 8/85 | 0.011*              | 3.28                 | 1.31–8.19             | 0.003**             | 4.31                 | 1.65–11.28            |
| Adjuvant therapy (no/yes)             | 47/46 | 0.012*              | 0.58                 | 0.38–0.89             | 0.009**             | 0.50                 | 0.30–0.84             |
| Palliative chemotherapy               | 32/56 | 0.88                | 0.68                 | 0.43–1.06             | Not included        |                     |                      |
| Percentage of PDAC SULF2 +ve          |     |                     |                      |                       |                     |                      |                      |
| 0                                     | 3   | 0.123               |                      |                       | Not included        |                     |                      |
| 1–25                                  | 34  | 0.057               | 6.94                 | 0.95–51.0             |                      |                     |                      |
| 26–50                                 | 17  | 0.080               | 6.12                 | 0.81–46.5             |                      |                     |                      |
| 51–75                                 | 17  | 0.084               | 5.99                 | 0.79–45.6             |                      |                     |                      |
| 76–100                                | 22  | 0.024*              | 10.3                 | 1.35–76.3             |                      |                     |                      |
| SULF2 intensity                       |     |                     |                      |                       |                     |                      |                      |
| None                                  | 3   | 0.008**             |                      | (0.066)               | (0.066)             |                      |                      |
| Weak                                  | 15  | 0.045*              | 7.96                 | 1.04–60.7             | (0.114)             | (0.067–42.0)        |                      |
| Moderate                              | 38  | 0.103               | 5.25                 | 0.72–38.5             | (0.174)             | (0.054–30.1)        |                      |
| Strong                                | 37  | 0.022*              | 10.4                 | 1.41–76.2             | (0.058)             | (0.093–53.0)        |                      |
| Combined SULF2 score                  |     |                     |                      |                       |                     |                      |                      |
| 0                                     | 3   | 0.028*              |                      | (0.084)               | (0.084)             |                      |                      |
| 2                                     | 9   | 0.066               | 6.97                 | 0.88–55.3             | (0.164)             | (0.049–37.3)        |                      |
| 3                                     | 8   | 0.084               | 6.33                 | 0.76–51.2             | (0.211)             | (0.384)             | (0.47–31.7)          |
| 4                                     | 35  | 0.055               | 7.04                 | 0.96–51.7             | (0.124)             | (0.484)             | (0.65–36.3)          |
| 5                                     | 13  | 0.127               | 4.94                 | 0.63–38.4             | (0.194)             | (3.84)              | (0.50–31.9)          |
| 6                                     | 13  | 0.040*              | 8.47                 | 1.10–65.3             | (0.089)             | (6.00)              | (0.76–47.3)          |
| 7                                     | 12  | 0.006**             | 17.9                 | 2.27–142.3            | (0.021)*            | (11.65)             | (1.44–94.1)          |
| Combined SULF2 score                  |     |                     |                      |                       |                     |                      |                      |
| 0–5 (lower) vs 6–7 (high)             | 68/25 | 0.002**             | 2.21                 | 1.35–3.61             | 0.004**             | 2.10                 | 1.26–3.54            |
| Tumour stromal SULF2                  |     |                     |                      |                       |                     |                      |                      |
| None                                  | 29  | 0.861               |                      |                       | Not included        |                     |                      |
| Focal                                 | 54  | 0.644               | 1.12                 | 0.70–1.78             |                      |                     |                      |
| Marked                                | 10  | 0.647               | 1.19                 | 0.57–2.46             |                      |                     |                      |
| Stromal SULF2 (outside)               |     |                     |                      |                       |                     |                      |                      |
| None                                  | 17  | 0.047*              |                      |                       |                     |                      |                      |
| Focal                                 | 48  | 0.103               | 0.62                 | 0.35–1.01             | 0.377               | (0.059–2.10)        |                      |
| Marked                                | 28  | 0.775               | 1.09                 | 0.59–2.01             | 0.224               | 1.51                 | (0.78–2.91)          |

Abbreviations: CI = confidence interval; HR = hazard ratio; PDAC = pancreatic ductal adenocarcinoma; SULF = sulfatase; TNM = tumour node metastases. The multivariate Cox regression analysis included variables (highlighted in bold) with a P value  0.05 in univariate analyses. The combined bivariate SULF2 score comparing low (0–5) vs high (6–7) was entered into the analysis shown. Data in brackets show similar multivariate analyses entering either SI score or combined SULF2 score with ordinal groups instead. For categorical variables, the comparator was the first listed variable. HR and 95% lower and upper CI are shown for all variables. *P<0.05; **P<0.01.
The major focus for biomarker discovery in PDAC has therefore been the identification of novel prognostic biomarkers to inform clinical decision making. For example, to identify poor prognosis patients that might be suitable for experimental therapy in the primary setting, and those with a relatively favourable prognosis in whom more aggressive conventional treatments may be appropriate. A large number of conventional clinical, circulating and pathological factors (Giovannazzo et al., 2012; Winter et al., 2013; Bilici, 2014; Jazieh et al., 2014; Lamarca and Felu, 2014), as well as genomic and proteomic parameters (Marengo and Robotti, 2014), have been the subject of biomarker discovery research in PDAC. However, the glycoprotein CA19–9, the only FDA approved biomarker for PDAC, is the sole factor in widespread routine clinical use. Serum CA19–9 has utility as a diagnostic, prognostic and surrogate response biomarker, but it has well known limitations and its precise value in these settings remains the subject of debate (Bilici, 2014; Jazieh et al., 2014).

Thus far, meta-analyses of immunohistochemical prognostic biomarker studies in PDAC have identified VEGF levels (Ansari et al., 2011; Smith et al., 2011), consistent with a role for the growth factor in tumour angiogenesis and hence progression and prognosis. More recently a meta-analysis assessing expression of the chemokine receptor CXCR4 supported an association between metastatic disease and overall survival in patients with PDAC (Krieg et al., 2015). Both of these prognostic biomarkers offer links to the molecular and cellular pathology of PDAC in a way that could ultimately be exploited therapeutically. As yet, however, these hopes have not been realised and there remains a need for additional prognostic biomarkers, preferably ones which may be predictive of responses to novel therapies.

Building on a previous study reporting an increase in SULF2 detected immunohistochemically in 4 of 7 patient PDAC samples relative to non-tumour tissues (Nawroth et al., 2007), the current study has identified expression of the extracellular endosulfatase SULF2 as poor prognostic biomarker in PDAC that is related to tumour stage, vascular invasion, radiological progression and overall survival. By controlling the degree of sulfation of extracellular HSPGs, and hence growth factor binding and signalling, SULF2 can regulate a number of growth factors that have been implicated in PDAC pathobiology, notably VEGF, Wnts and TGF-β (Nawroth et al., 2007; Rosen and Lemjabbar-Alaoui, 2010). Importantly, SULF2 is at the interface between the PDAC cell and the tumour microenvironment, and the importance of local tumour-host cell interactions in cancer biology is increasingly recognised. Hence the biological function of SULF2, its involvement in pathways of known importance in PDAC pathogenesis and evidence that SULF2 is a prognostic biomarker, presented here, all identify this enzyme as important in PDAC patient survival.

In the subgroup of patients who received adjuvant chemotherapy ($n = 46$), age, sex and tumour features had no impact on survival. Again, however, higher SULF2-positive PDAC cells or strong SULF2 SI were associated with poorer outcomes. Using the combined IHC score, median survival was 25 months in those with lower SULF2 (score 0–5) vs 12 months in those with a high SULF2 (score 6–7; $P = 0.001, HR 3.23, CI 1.59–6.57$) as shown in Figure 2B.

**DISCUSSION**

The development of personalised or precision medicine for cancer therapy requires the discovery, qualification and clinical use of prognostic and predictive biomarkers. In PDAC the most common driver genes with recurrent mutations; $KRAS$, $SMAD4$, $TP53$ and $CDKN2A/p16$, cannot currently be exploited by available targeted therapies. Whole-genome sequencing has identified new candidates which may be druggable, but at low individual prevalence (Waddell et al., 2015). Hence predictive biomarkers that can be used to select specific treatments for individual patients have not had a major impact on the management of the disease, with systemic treatment being limited to single agent (e.g., gemcitabine) or combination (e.g., FOLFIRINOX) cytotoxic drug therapy.

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**DISCUSSION**

The development of personalised or precision medicine for cancer therapy requires the discovery, qualification and clinical use of prognostic and predictive biomarkers. In PDAC the most common driver genes with recurrent mutations; $KRAS$, $SMAD4$, $TP53$ and $CDKN2A/p16$, cannot currently be exploited by available targeted therapies. Whole-genome sequencing has identified new candidates which may be druggable, but at low individual prevalence (Waddell et al., 2015). Hence predictive biomarkers that can be used to select specific treatments for individual patients have not had a major impact on the management of the disease, with systemic treatment being limited to single agent (e.g., gemcitabine) or combination (e.g., FOLFIRINOX) cytotoxic drug therapy.

The major focus for biomarker discovery in PDAC has therefore been the identification of novel prognostic biomarkers to inform clinical decision making. For example, to identify poor prognosis patients that might be suitable for experimental therapy in the primary setting, and those with a relatively favourable prognosis in whom more aggressive conventional treatments may be appropriate. A large number of conventional clinical, circulating and pathological factors (Giovannazzo et al., 2012; Winter et al., 2013; Bilici, 2014; Jazieh et al., 2014; Lamarca and Felu, 2014), as well as genomic and proteomic parameters (Marengo and Robotti, 2014), have been the subject of biomarker discovery research in PDAC. However, the glycoprotein CA19–9, the only FDA approved biomarker for PDAC, is the sole factor in widespread routine clinical use. Serum CA19–9 has utility as a diagnostic, prognostic and surrogate response biomarker, but it has well known limitations and its precise value in these settings remains the subject of debate (Bilici, 2014; Jazieh et al., 2014).

Thus far, meta-analyses of immunohistochemical prognostic biomarker studies in PDAC have identified VEGF levels (Ansari et al., 2011; Smith et al., 2011), consistent with a role for the growth factor in tumour angiogenesis and hence progression and prognosis. More recently a meta-analysis assessing expression of the chemokine receptor CXCR4 supported an association between metastatic disease and overall survival in patients with PDAC (Krieg et al., 2015). Both of these prognostic biomarkers offer links to the molecular and cellular pathology of PDAC in a way that could ultimately be exploited therapeutically. As yet, however, these hopes have not been realised and there remains a need for additional prognostic biomarkers, preferably ones which may be predictive of responses to novel therapies.

Building on a previous study reporting an increase in SULF2 detected immunohistochemically in 4 of 7 patient PDAC samples relative to non-tumour tissues (Nawroth et al., 2007), the current study has identified expression of the extracellular endosulfatase SULF2 as poor prognostic biomarker in PDAC that is related to tumour stage, vascular invasion, radiological progression and overall survival. By controlling the degree of sulfation of extracellular HSPGs, and hence growth factor binding and signalling, SULF2 can regulate a number of growth factors that have been implicated in PDAC pathobiology, notably VEGF, Wnts and TGF-β (Nawroth et al., 2007; Rosen and Lemjabbar-Alaoui, 2010). Importantly, SULF2 is at the interface between the PDAC cell and the tumour microenvironment, and the importance of local tumour-host cell interactions in cancer biology is increasingly recognised. Hence the biological function of SULF2, its involvement in pathways of known importance in PDAC pathobiology and evidence that SULF2 is a prognostic biomarker, presented here, all identify this enzyme as important in PDAC patient survival.
SULF2 in pancreatic ductal adenocarcinoma

ACKNOWLEDGEMENTS

HLR and this data generated in this project were supported by the Faculty of Medicine, Newcastle University and the Newcastle upon Tyne NHS Foundation Trust. SA was in part supported by a personal award from Damasus University. GB and DRW were supported by a Cancer Research UK Drug Discovery Programme Grant. Thanks also to Dr Benjamin Marrow who helped to collect clinical data.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies this paper on British Journal of Cancer website (http://www.nature.com/bjc)