© 2018. Published by The Company of Biologists Ltd | Journal of Cell Science (2018) 131, jcs220640. doi:10.1242/jcs.220640

RESEARCH ARTICLE

CD151 regulates expression of FGFR2 in breast cancer cells via PKC-dependent pathways

Rafal Sadej[1,*], Xiaohong Lu[2,*], Lukasz Turczyk[1], Vera Novitskaya[2], Andrea F. Lopez-Clavijo[3], Radzisław Kordek[4], Piotr Potemski[4], Michael J. O. Wakelam[3], Hanna Romanska-Knight[4,‡] and Fedor Berditchevski[2,‡]

ABSTRACT

Expression of the tetraspanin CD151 is frequently upregulated in epithelial malignancies and correlates with poor prognosis. Here, we report that CD151 is involved in regulation of the expression of fibroblast growth factor receptor 2 (FGFR2). Depletion of CD151 in breast cancer cells resulted in an increased level of FGFR2. Accordingly, an inverse correlation between CD151 and FGFR2 was observed in breast cancer tissues. CD151-dependent regulation of the FGFR2 expression relies on post-transcriptional mechanisms involving HuR (also known as ELAVL1), a multifunctional RNA-binding protein, and the assembly of processing bodies (P-bodies). Depletion of CD151 correlated with inhibition of PKC, a well-established downstream target of CD151. Accordingly, the levels of dialcylglycerol species were decreased in CD151-negative cells, and inhibition of PKC resulted in the increased expression of FGFR2. Whereas expression of FGFR2 itself did not correlate with any of the clinicopathological data, we found that FGFR2^{-}/CD151^{+} patients were more likely to have developed lymph node metastasis. Conversely, FGFR2^{-}/CD151^{-} patients demonstrated better overall survival. These results illustrate functional interdependency between CD151 complexes and FGFR2, and suggest a previously unsuspected role of CD151 in breast tumorigenesis.

KEY WORDS: CD151, FGFR2, Breast cancer, Tetraspanin, PKC

INTRODUCTION

Tetraspanins represent a large group of four transmembrane domain proteins with diverse biological activities (Berditchevski and Rubinstein, 2013). At the biochemical level, tetraspanins are thought to function as the main structural blocks and organizers of distinct microdomains on the plasma membrane, which also include transmembrane receptors (e.g. integrins, receptor tyrosine kinases) and cytoplasmic signalling proteins. Analyses of clinical material have suggested that several tetraspanin proteins may be involved in the development and metastatic progression in various cancer types (Hemler, 2014).

[1]Department of Molecular Enzymology, Intercollegiate Faculty of Biotechnology, University of Gdansk and Medical University of Gdansk, Abrahama 58, 80-307 Gdańsk, Poland. [2]Institute of Cancer and Genomic Sciences, The University of Birmingham, Edgbaston, Birmingham B15 2TT, UK. [3]Babraham Institute, Cambridge CB22 3AT, UK. [4]Department of Pathology and Chemotherapy, Medical University of Łódź, 92-213 Łódź, Poland.
*These authors contributed equally to this work

‡Authors for correspondence (f.berditchevski@bham.ac.uk; hanna.romanska@gmail.com)

H.R., 0000-0002-2328-710X; F.B., 0000-0003-0911-834X

Received 22 May 2018; Accepted 17 September 2018

Expression of the tetraspanin CD151 is elevated in various types of breast cancer, and this is correlated with poor prognosis and overall survival in breast cancer patients (Kwon et al., 2012; Novitskaya et al., 2014). Experiments involving cell lines and animal models suggested that pro-tumorigenic and pro-metastatic functions of CD151 are likely to be dependent on its ability to form complexes with laminin-binding integrin receptors (i.e. α6β1, α3β1 and α6β4) and coordinate integrin-dependent signalling networks in the context of receptor tyrosine kinases (Sadej et al., 2014). Specifically, CD151 is known to regulate integrin ligand binding and post-adhesion signalling, including activation of small GTPases (Rho, Rac and Cdc42), FAK, Akt and Erk1/2 (Sadej et al., 2014). The association with integrins is also important for CD151-dependent regulation of cellular responses to growth factors and inhibitory drugs that target receptor tyrosine kinases (RTKs) in cancer cells (Deng et al., 2012; Novitskaya et al., 2014; Sadej et al., 2010). Cross-talk between integrins and RTKs has been shown to involve classical protein kinase C (PKC), well-established molecular partners for several tetraspanin proteins including CD151 (Li et al., 2013). In addition to regulating integrin function, CD151 may influence the metastatic potential of cancer cells indirectly via E-cadherin-based cell–cell adhesion complexes (Johnson et al., 2009; Shigeta et al., 2003), or through the increased production of extracellular matrix-degrading enzymes (e.g. matrix metalloproteases) (Hasegawa et al., 2007).

FGFR2 is a member of a receptor tyrosine kinase subfamily that also includes FGFR1, FGFR3 and FGFR4 (Kelleher et al., 2013). There is increasing evidence that the FGF–FGFR2 signalling axis plays an important role in breast cancer. Genome-wide analysis has identified a number of single-nucleotide polymorphic (SNP) variants in intron 2 of the FGFR2 gene that are associated with a higher incidence of risk of breast cancer (Cui et al., 2016), and in particular, in patients positive for hormonal receptors and negative for Her2 (also known as ErbB2) (Cox et al., 2016). Several transcription factors (e.g. FoxA1, Oct1 and E2F1) have been shown to bind differentially to the high-risk alleles and, therefore, might be responsible for elevated expression of FGFR2 in patients carrying these alleles (Robbez-Masson et al., 2013). Furthermore, gene amplification and overexpression of FGFR2 in breast cancer tissues has been also described, particularly in the triple-negative (i.e. ER^{-}/PR^{-}/ErbB2-negative) breast cancers (Turner et al., 2010). Accordingly, overexpression and FGFR2 inhibitor studies using human cell models supported the pro-tumorigenic function of FGFR2 in breast cancer (Bai et al., 2010; Sommer et al., 2016). By contrast, activation of FGFR2 in mouse primary mammary epithelial cells resulted in activation of apoptosis (Xian et al., 2007). It was also reported that FGFR2 inhibited epithelial-to-mesenchymal transition and attenuated growth of human breast cancer xenografts *in vivo* (Tarkkonen et al., 2012).

Journal of Cell Science (2018) 131, jcs220640. doi:10.1242/jcs.220640

Here, we describe a new link between the tetraspanin CD151 and FGFR2 in breast cancer. Specifically, we found that CD151 controls the expression level of FGFR2 using a pathway that is independent of proteolytic degradation or transcriptional regulation. Instead, it involves the assembly of processing bodies (P-bodies) and PKC-dependent signalling pathways. Importantly, the inverse correlation between expression of CD151 and FGFR2 in cellular models of breast cancer was also observed in breast cancer tissues, thus emphasizing the importance of our findings for future translational studies.

RESULTS

CD151 regulates expression of FGFR2

While analysing the expression of FGF receptors and tetraspanin proteins in a panel of breast cancer cell lines, we noticed a tendency for an inverse correlation between the levels of FGFR2 and CD151 (Fig. 1A). In these experiments, we detected no correlation between expression levels of CD151 and FGFR1, on one hand, or between FGFR2 and several other tetraspanins (i.e. CD9, CD63, CD81 and CD82), on the other (Fig. 1A and data not shown). In agreement with this observation, stable silencing of CD151 [herein depletion or negative for expression is denoted by '(−)' where wild-type positive expression is denoted by '(+)'], but not CD63 or CD81, resulted in increased FGFR2 levels in HB2 cells, a DCIS-like mammary epithelial cell line, as well as in SKBR3 and MCF7 cells – two widely used breast cancer cell models (Fig. 1B and data not shown). The re-expression of CD151 in HB2/CD151(−) cells reverted the FGFR2 protein level to that observed in HB2/CD151(+) cells, thus further excluding the off-target effect of the CD151 shRNA construct (Fig. 1C). An increased level of FGFR2 was also observed when CD151 was targeted with an alternative siRNA in transient knockdown experiments (Fig. S1). Importantly, knockdown of CD151 had no effect on expression levels of FGFR1 or FGFR4 (FGFR3 was not detectable), emphasizing the specific relationship between CD151 and FGFR2 (Fig. 1B).

Previous studies have demonstrated that most of CD151-dependent regulation of cellular functions involves laminin-binding integrins (α3β1 and α6 integrins) (Sadej et al., 2014). Surprisingly, we found that depletion of these integrins in HB2 cells, either separately or in combination, had no effect on the expression level of FGFR2 (Fig. 1D), thus demonstrating that the CD151-dependent regulation of the FGFR2 expression does not involve integrins.

Depletion of CD151 accentuates the responses of mammary epithelial cells to FGFs

The initial proliferation experiments demonstrated that HB2/CD151(+) and HB2/CD151(−) cells responded similarly to FGF2 and FGF9 when cultured under standard 2D conditions (Fig. S2 and data not shown). In contrast, the growth response of CD151-depleted cells to FGFs was more robust when cells were placed in a 3D laminin-rich extracellular matrix (3D-lrECM) (Fig. 2A). Furthermore, we noticed that FGF-treated HB2/CD151(−) colonies lost their smooth 'ball-like' morphology and appeared as disorganized aggregates of cells (Fig. 2A). Importantly, re-expression of wild-type CD151 reversed the proliferative and morphological phenotypes of HB2/CD151(−) cells (Fig. 2B). Similarly, FGF-induced proliferation of CD151-depleted SKBR3 cells in 3D-lrECM was more pronounced when compared to the control, SKBR3/CD151(+) cells (Fig. 2C). Analysis of the

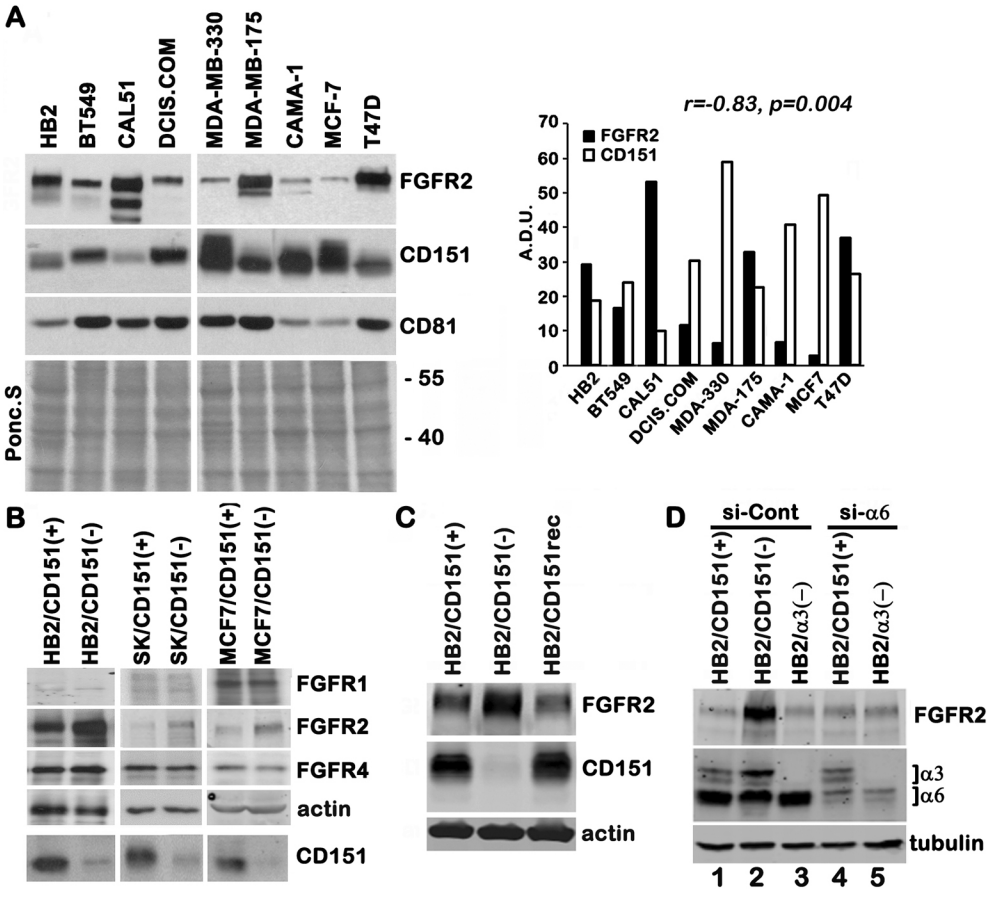


Fig. 1. CD151 regulates expression of FGFR2 gene. (A) An inverse correlation between CD151 and FGFR2 expression in a panel of breast cancer cell lines. Protein lysates (20 µg) were resolved by SDS-PAGE and expression levels of CD151, CD81 and FGFR2 were analysed by western blotting. Membranes were stained with Ponceau S to control for the amounts of loading material. Right panel displays arbitrary density units (A.D.U.) for FGFR2 and CD151 signals. The Pearson's correlation coefficient (*r*) was calculated using Excel 2010. (B) Knockdown of CD151 in mammary epithelial cells results in a specific increase of FGFR2 expression without affecting other FGF receptors. Expression of FGF receptors was analysed as described in A. (C) Stable re-expression of shRNA-resistant form of CD151 in CD151-depleted cells (HB2/CD151rec) reversed FGFR2 expression in HB2 cells. (D) Silencing of α3 and α6 integrins does not affect the expression level of FGFR2. Expression of FGFR2 was analysed in HB2 cells depleted of CD151 (lane 2), α3 integrin subunit (lane 3), α6 integrin subunit (lane 4) and (α3+α6) integrin subunits (lane 5). HB2/CD151(+) cells were used as a control. The middle panel shows expression levels of α3 and α6 integrin subunits.

Journal of Cell Science

Journal of Cell Science (2018) 131, jcs220640. doi:10.1242/jcs.220640

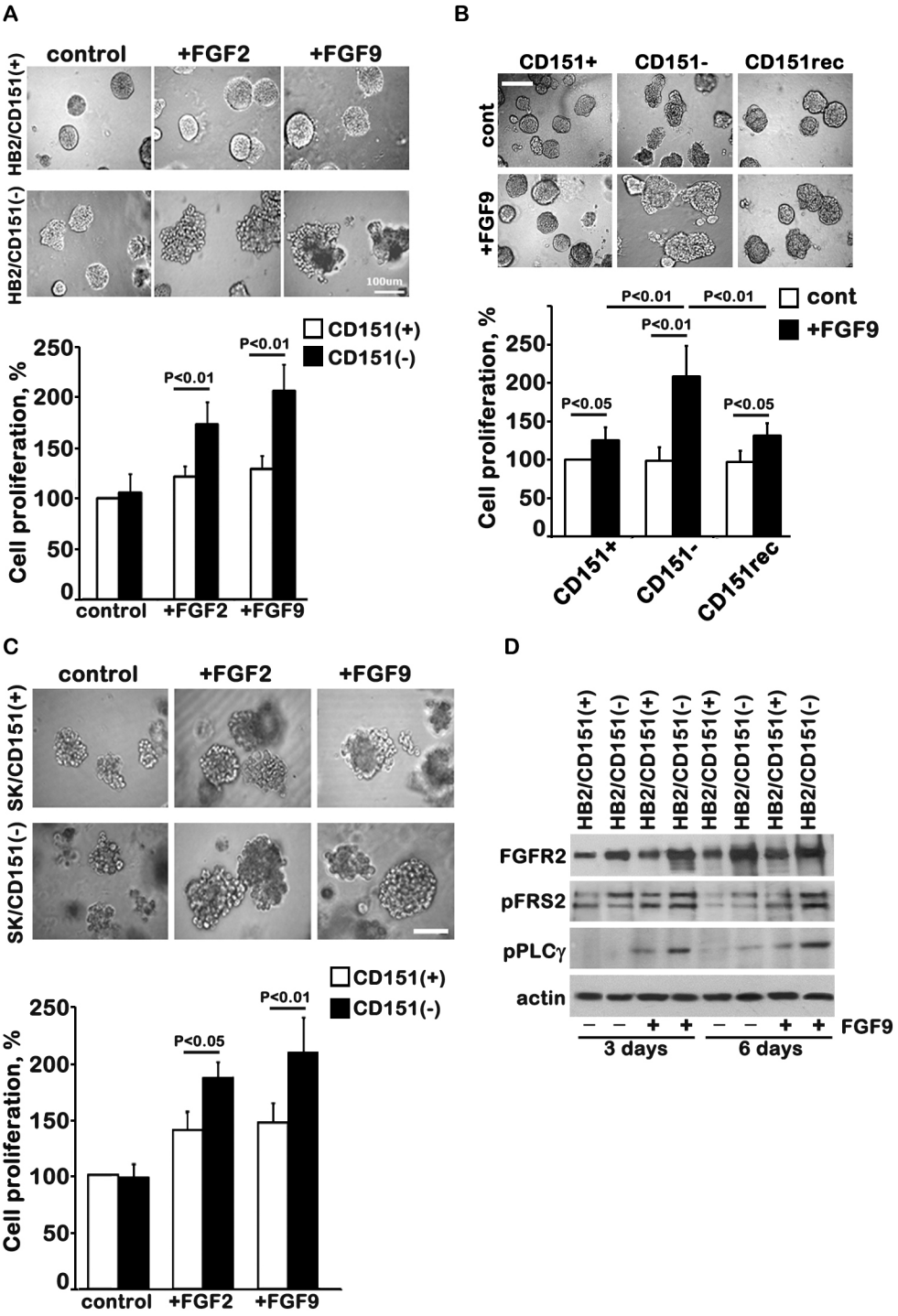


Fig. 2. CD151 regulates cellular responses to FGFs. HB2 (A,B) and SKBR3 (C) CD151(+) and CD151(–) cells were embedded in 3D lrECM and grown for 8 days in the presence of FGF2 and FGF9 (50 ng/ml). Representative images are shown. Proliferation was measured by using WST-1 reagent as described in the Materials and Methods, and shown as histograms in which bars represent averaged results (mean±s.d.) of three independent experiments. *P* values were calculated using two-tailed *t*-test and are indicated on graphs. Proliferation is presented as percentage of the control, CD151 cells grown under standard culturing conditions. (D) Activation (phosphorylation indicated by 'p' prefix) of FGFR2-dependent signalling pathways in cells grown under standard culturing conditions was analysed by western blotting. Shown representative results of one of three independent experiments. Scale bars: 100 µm.

FGF-triggered signalling in HB2 cells growing in 3D-lrECM revealed higher levels of phosphorylation of FRS2 and PLCγ (specific downstream effectors of FGFR-triggered signalling) in CD151-depleted cells (Fig. 2D). Taken together, these results demonstrate that elevated FGFR2 levels in CD151-depleted cells translate into more-prominent responses of breast cancer cells to FGFs.

CD151 regulates expression of FGFR2 at the post-transcriptional level

To investigate how CD151 controls the expression of FGFR2, we initially examined the effect of inhibitors of various proteolytic pathways. In these experiments, we found that treatment of HB2 cells with inhibitors of matrix metalloproteases (GM6001, Batimastat), serine proteases (leupeptin), aspartic proteases (pepstatin), calpains (calpastatin) and caspases (Z-VAD-FMK) did not change expression levels of FGFR2 either in CD151-positive or CD151-negative cells (Fig. S3 and data not shown). Likewise, bafilomycin A1 and MG132, general inhibitors of endosomal/lysosomal and proteosomal degradation have no notable effect on FGFR2 levels in HB2/CD151(+) and HB2/CD151(–) cells (Fig. S3 and data not shown). Therefore, we concluded that CD151-dependent regulation of the FGFR2 expression does not involve common proteolytic pathways.

Journal of Cell Science (2018) 131, jcs220640. doi:10.1242/jcs.220640

In further experiments, we compared the levels of *FGFR2* mRNA in the control and CD151-depleted cells. Several probes covering various parts of *FGFR2* mRNA were used in quantitative RT-PCR experiments to account for possible variations in *FGFR2* splicing. There were no differences observed between the control and CD151-depleted cells in these experiments (Fig. S4), thus ruling out the regulation at the level of mRNA.

Transcribed mRNAs bind a diverse range of nuclear proteins that facilitate their export to the cytoplasm where the mRNP complexes are either immediately translated or stored in various types of cytoplasmic granules (Buchan, 2014). Thus, we examined whether depletion of CD151 affected the assembly of stress granules (SGs), P-bodies and GW bodies, the three most common types of RNA-containing granules in eukaryotic cells. In these experiments, we found that the number of P-bodies was noticeably reduced in CD151-negative HB2 and SKBr3 cells (Fig. 3A). By contrast, the distribution of TIA-1 (a SG marker) and GW182 (also known as TNRC6A; a marker for GW bodies) was comparable in HB2/CD151(+) and HB2/CD151(−) (results not shown). To examine the role of P-bodies in FGFR2 expression directly, we silenced the expression of EDC4 and Pat1b, which have been shown to regulate the assembly of P-bodies (Stalder and Mühlemann, 2009; Ozgur et al., 2010). As illustrated in Fig. 3B, depletion of either protein increased FGFR2 levels. Therefore, we concluded that the elevated expression of FGFR2 in CD151-negative cells is likely to involve P-bodies.

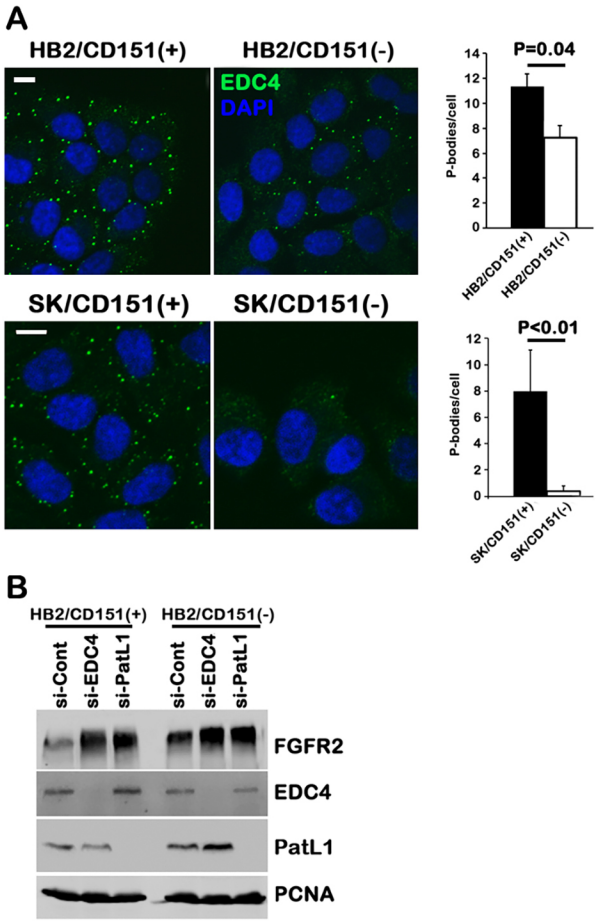


Fig. 3. The effect of CD151 depletion on the formation of P-bodies, and the role of P-bodies in the expression of FGFR2. (A) The number of P-bodies is decreased in CD151-depleted cells. Cells were grown under standard culturing conditions for 48 h before staining with the anti-EDC4 antibody to reveal P-bodies. Representative images are shown. Numbers of P-bodies was quantified in at least 70–100 cells. Scale bars: 10 µm. The graphs show averaged results (mean±s.d.) of number of P-bodies per cell. *P* values were calculated using two-tailed *t*-test. (B) Knockdown of the key components of P-bodies (EDC4 and PatL1) increases FGFR2 expression. Cells were depleted of EDC4 and PatL1 using specific siRNAs. si-Cont, control siRNA. The expression of FGFR2 was assessed by western blotting 72 h after transfection. Results of one of two independent experiments are shown.

The mRNA-binding protein ELAVL-1/HuR is involved in regulation of FGFR2 expression

Nuclear/cytoplasmic proteins TIA-1, TIAR (also known as TIAL1), hnRNP-C1, hnRNP-C2 (hnRNP-C1/C2) and ELAVL-1 (also known as HuR) have been shown to bind *FGFR2* mRNA and are known to be functionally linked to P-bodies (Izquierdo, 2010; Del Gatto-Konczak et al., 2000; Hubstenberger et al., 2017; Stoecklin and Kedersha, 2013; Lebedeva et al., 2011). Therefore, we examined whether these proteins can be involved in CD151-dependent regulation of expression of FGFR2 protein in breast cancer cells. Total expression levels and nuclear versus cytoplasmic accumulation of these proteins were comparable in CD151-positive and CD151-negative cells (Fig. 4A). Knockdown of ELAVL-1 resulted in an increased level of FGFR2 protein in HB2 and SKBr3 cells (Fig. 4B). By contrast, the expression level of FGFR1 was not affected (Fig. S5A). Furthermore, depletion of TIA-1, TIAR or hnRNP-C1/C2 had no or a minimal effect on the expression levels of FGFR2 in either CD151-positive or CD151-negative cells (Fig. S5B).

CD151-dependent regulation of FGFR2 expression involves PKC

To examine the molecular mechanisms that could link CD151 with the activity of ELAVL-1 towards FGFR2, we analysed signalling pathways that are known to be regulated by tetraspanins, on one hand, and affect the function of ELAVL-1, on the other. Specifically, we compared activation of Src, p38 MAPK proteins and PKC in the control and CD151-depleted HB2 cells. As illustrated in Fig. 5A, the levels of active Src and p38 kinases were comparable in HB2/CD151(+) and HB2/CD151(−) cells, thereby ruling out the involvement of these proteins in CD151-dependent regulation of FGFR2 expression. CD151 has been shown to associate with classical protein kinases C (cPKC) (Zhang et al., 2001). Interestingly, we found that the phosphorylation levels of several cellular proteins recognized by the anti-phospho-(Ser) PKC substrate antibody were higher in CD151(+) HB2 cells (Fig. 5B), thus indicating that depletion of CD151 results in a lower basal level of activation of PKC. Importantly, treatment of cells with bisindolylmaleimide I (BIM I), a highly specific PKC inhibitor, increased FGFR2 levels in both HB2 and SKBr3 cells (Fig. 5C). Interestingly, treatment of HB2/CD151(+) cells with BIM I had no effect on the assembly of P-bodies (Fig. S6). These results suggest that, although activation of PKC is important for regulation of FGFR2 expression by CD151, other pathways are responsible for the effect of CD151 on the assembly of P-bodies.

It has been previously reported that when activated by phorbol 12-myristate 13-acetate (PMA) classical PKC form complexes with various tetraspanin proteins including CD151 (Termini and Gillette, 2017). Thus, increased activation of PKC in CD151(+) breast cancer cells might have been due to the association with tetraspanin microdomains (or, more specifically, with CD151-containing complexes). However, in accordance with previous studies, PKC was not a part of the CD151 complexes purified from cells grown under standard culturing conditions (Fig. S7). We therefore considered whether increased PKC activation in CD151(+) cells is

Journal of Cell Science (2018) 131, jcs220640. doi:10.1242/jcs.220640

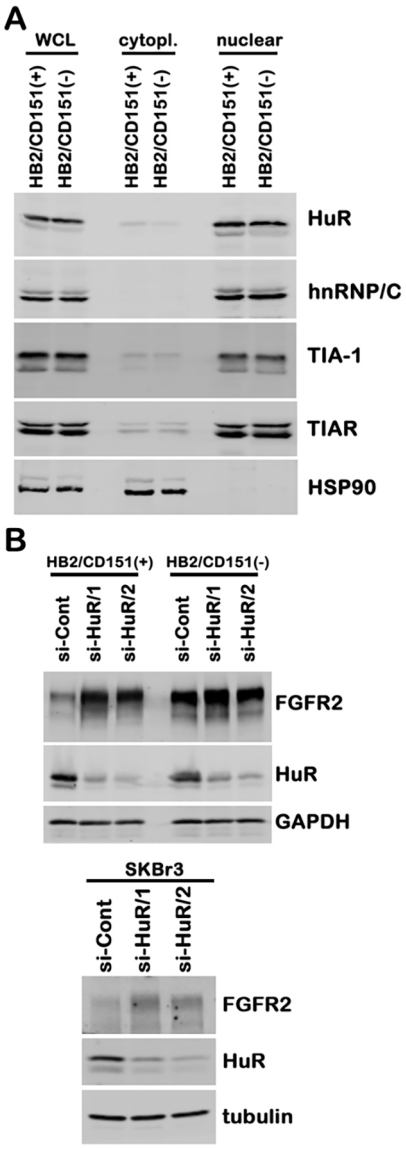


Fig. 4. The role of ELAVL-1 in CD151-dependent regulation of FGFR2 expression. (A) Expression levels and distribution of mRNA-binding proteins in HB2 cells was analysed by western blotting using specific antibodies. WCL, whole cell lysates; cytopl. and nuclear, cytoplasmic and nuclear fractions of the proteins, respectively. Note, HSP90 is only found in the cytoplasmic fractions. (B) The effect of ELAVL-1 (HuR) siRNA (two different siRNAs indicated by si-HuR/1 and siHuR/2) knockdown on the FGFR2 expression in HB2 and SKBr3 cells. The expression of FGFR2 was assessed by western blotting 72 h after transfection. Shown results of one of the two independent experiments.

due to differences in the levels of diacylglycerol (DAG), the physiological activator of classical and novel PKC. A quantitative analysis of DAG species in HB2/CD151(+) and HB2/CD151(−) cells revealed that depletion of CD151 resulted in 1.5–2-fold decrease in the levels of various DAGs species with the most pronounced differences observed for 34:1, 36:1, 36:2 and 38:4 species (Fig. 5D). Importantly, the effect of CD151 depletion on these DAGs was specific, as we observed no differences in the levels of some other species (e.g. 32:1 and 34:2). Although differences in the levels of DAGs were not due to the activity of PLCγ (as monitored by the phosphorylation levels of Tyr783) (Fig. 5E), we found that depletion of CD151 resulted in changes in the intracellular distribution of the enzyme with most cells losing their prominent PLCγ-positive puncta/vesicles (Fig. 5F). Thus, we conclude that CD151-dependent regulation of PKC activation and, subsequently, expression of FGFR2 is controlled by the abundance of particular DAGs, which may be caused by changes in compartmentalization of PLCγ.

An inverse relationship between FGFR2 and CD151, but not the expression of FGFR2 alone, is associated with invasiveness of breast cancer

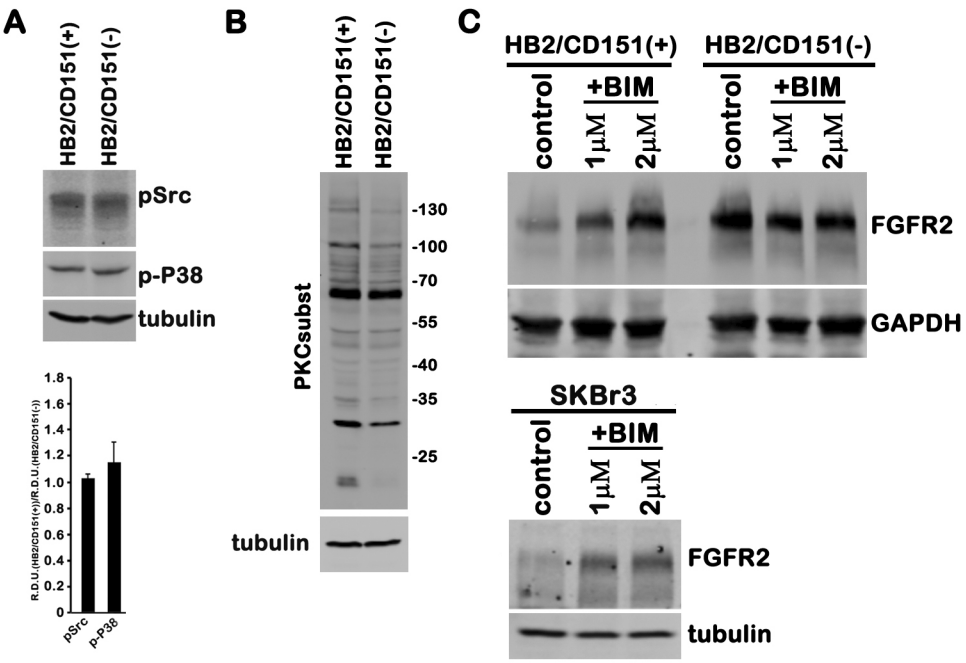
To examine links between FGFR2 and CD151 further, we analysed co-expression of these proteins in tissue samples from patients with invasive ductal carcinoma (IDC) (Table 1). In the majority of both normal mammary glands and tumours, expression of FGFR2 was highly heterogeneous. Various patterns of FGFR2 immunostaining, that is, cytoplasmic and/or membranous (39%; 65/166), nuclear (45%; 75/166) or ubiquitous (23%; 38/166) could be seen. There was no association between levels of FGFR2 immunoreactivity in distinct cellular compartments. A highly heterogeneous pattern of immunoreactivity was also observed when expression of FGFR2 was analysed in combination with immunoreactivity for CD151. An inverse relationship (i.e. expression of either FGFR2 or CDC151 but not both) between FGFR2 and CD151 expression was seen in many samples, with 51/166 (31%) being FGFR2(−)/CD151(+) and 38/166 (23%) being and FGFR2(+)/CD151(−), respectively. In 50/166 cases (30%) tumours were negative for both FGFR2 and CD151, while a FGFR2(+)/CD151(+) trait was found in a minority of samples (27/166; 16%). Importantly, a Kendall's test demonstrated a nonlinear negative correlation between cytoplasmic/membranous FGFR2 and CD151 (τ=0.14, P=0.029), thus supporting the inverse relationship between FGFR2 and CD151 levels in breast cancer cells. Expression of nuclear FGFR2 alone did not correlate with any of the tumour characteristics and cytoplasmic/membranous FGFR2 was significantly associated only with lack of hormone receptors (P=0.04) (Table 1). However, when assessed in combination with CD151, an inverse FGFR2/CD151 expression in tumour cells was significantly correlated with clinicopathological variables such as grade [FGFR2(+)/CD151(−) versus rest of the samples; P=0.012] and nodal status [FGFR2(−)/CD151(+) versus rest of the samples; P=0.037] (Tables 1 and 2). The FGFR2(−)/CD151(+) phenotype was also more frequently seen in the triple-negative subgroup of the studied cohort (P=0.039) (Table 1). Expression of FGFR2 alone (either cytoplasmic/membranous or nuclear) was of no prognostic value (Fig. 6A). Interestingly, its absence from the cell, when combined with lack of CD151 expression [FGFR2(−)/CD151(−)], not only inversely correlated with nodal status (P=0.013) and HER2 (P=0.014) (Tables 1 and 2), but also proved a good prognostic indicator (P=0.024) (Fig. 6B), albeit not independent (results of multivariate analysis not shown).

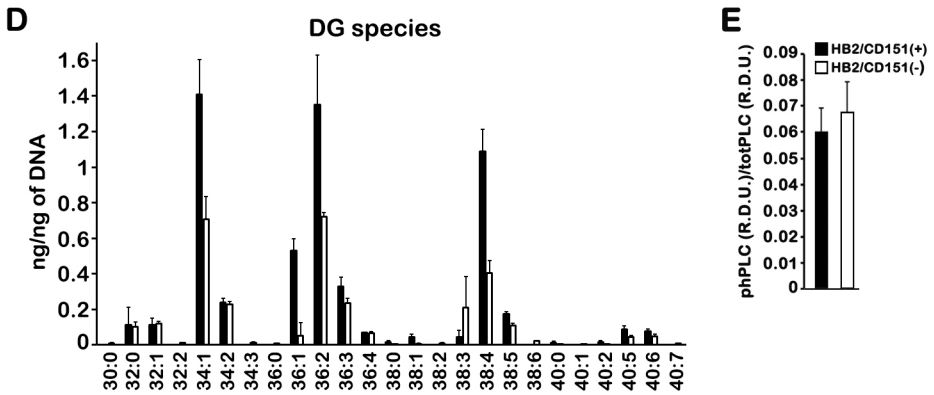
DISCUSSION

Dysregulation of the FGF–FGFR2 signalling network is thought to play an important role in various epithelial malignancies (Katoh and Nakagama, 2014). Point mutations, gene amplification, tumour-associated alternative splicing and gene rearrangements can lead to changes in FGFR2 function (Fearon et al., 2013; Katoh, 2008, 2009; Wu et al., 2013). Here, we show for the first time that the expression of FGFR2 in breast cancer cells is controlled by CD151-based signalling complexes. Importantly, we establish that CD151-dependent post-transcription regulation of FGFR2 expression is specific (i.e. CD151 does not regulate expression of FGFR1 or FGFR4) and involves PKC.

Importantly, in agreement with our *in vitro* observation, an inverse correlation between expression of CD151 and FGFR2 was also seen

Journal of Cell Science (2018) 131, jcs220640. doi:10.1242/jcs.220640





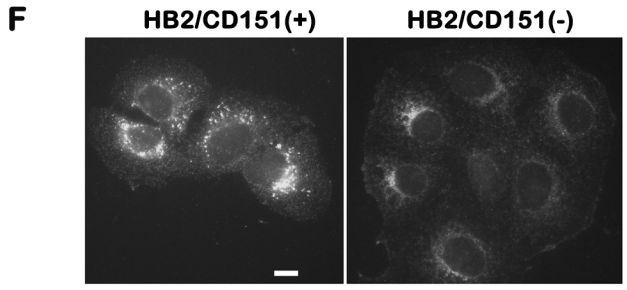


Fig. 5. The role of PKC in CD151-dependent regulation of the FGFR2 expression. (A) Activation of p38 MAPKs and Src (phosphorylation indicated by 'p' prefix) in cells grown under standard culturing conditions was analysed by western blotting using appropriate phosphorylation-specific antibodies. Histogram shows compilation of densitometric measurements of three independent experiments. R.D.U., relative density units. (B) Activation of PKC in cells grown under standard culturing conditions was analysed by western blotting using anti-phospho-(Ser) PKC substrate antibody. (C) Cells were grown under standard culturing conditions in the presence of BIM-I for 72 h and the expression of FGFR2 was assessed by western blotting. Shown is a representative image of three independent experiments. (D) Determination of DAG species (DG species) present. The concentrations of individual DAG molecular species were determined by mass spectrometry in HB2/CD151(+) (closed bars) and HB2/CD151(−) cells (open bars), and normalized to cellular DNA content. Results are mean±s.d. (n=4). (E) Activation of PLCγ was assessed by western blotting using anti-phospho-PLCγ (Tyr783) antibody in three independently prepared cellular lysates. Shown are the mean±s.d. ratio for the signal intensities of phPLCγ to total PLCγ. (F) Distribution of PLCγ in HB2/CD151(+) and HB2/CD151(−) cells. Cells were grown under standard culturing conditions for 48 h before staining with the anti-PLCγ antibody. Representative images are shown. Scale bar: 10 µm.

in the tumour tissues. These results imply that the pathway(s) that link expression of these proteins are also operational *in vivo*. Expression of FGFR2 alone did not have any prognostic value, which confirms our recent observations (Czaplinska et al., 2016). Although hormone receptors were the only clinicopathological feature associated with FGFR2, in contrast to the previous study, its relationship was inverse. The discrepancy between these results may reflect differential roles played by FGFR2 in distinct subtypes of breast carcinoma, as unlike findings shown by Czaplinska and colleagues, our analysis was performed on a histologically homogeneous group of specimens (i.e. invasive ductal carcinoma). In the patients with inverse correlation of CD151 and FGFR2 expression, only those that expressed high levels of CD151 [i.e. FGFR2(−)/CD151(+)] demonstrated correlation with lymph node involvement. Interestingly, a cohort of FGFR2(+)/CD151(+) patients did not show such correlation, which suggests that the presence of FGFR2 may suppress prometastatic activity of CD151. Indeed, this and previous studies (Klosek et al., 2009; Kwon et al., 2012) have revealed a highly significant correlation between the expression of CD151 and metastasis to the lymph nodes. Conversely, it was only in the absence of FGFR2 expression that CD151-negative cancer cells [FGFR2(−)/CD151(−) cohort] were less likely to be found in the lymph nodes. These results suggest that the proposed prometastatic function of CD151 in breast and, possibly, other cancers should be assessed in the context of FGFR2 expression.

Journal of Cell Science (2018) 131, jcs220640. doi:10.1242/jcs.220640

Table 1. Relationship between clinicopathological features and expression of (1) FGFR2 alone and (2) FGFR2 in association with CD151

Feature	FGFR2(+) (*n*=65) vs rest (*n*=101)	FGFR2(+)/CD151(−) (*n*=38) vs rest (*n*=128)	FGFR2(−)/CD151(+) (*n*=55) vs rest (*n*=111)	FGFR2(+)/CD151(+) (*n*=27) vs rest (*n*=139)	FGFR2(−)/CD151 (*n*=50) vs rest (*n*=116)
Tumour size	0.131	0.344	0.164	0.487	0.839
Nodal status	0.724	0.933	**0.037**	0.574	**0.013**
Stage	0.510	0.366	0.114	>0.999	0.369
Grade	0.095	**0.012**	0.102	0.510	0.895
HER2	0.197	0.771	0.281	0.171	**0.014**
ER/PR	**0.040**	0.221	0.066	0.185	0.736
TN	0.293	0.595	**0.039**	0.437	0.333

ER, oestrogen receptor; PR, progesterone receptor. Statistically significant correlation is shown in bold.

We discovered that CD151-dependent regulation of FGFR2 expression occurs at the post-transcriptional level via the mechanisms involving P-bodies. P-bodies are ribonucleoprotein-containing cytoplasmic aggregates that are known to control mRNA stability and translation (Anderson et al., 2015). The involvement of P-bodies adds a new layer of complexity to regulation of FGFR2 expression. Our data strongly suggest that ELAVL-1 is likely to be a critical downstream component of the CD151-dependent signalling network that is targeting FGFR2 mRNA. In other experiments, we found that FGFR2 is not the only target for ELAVL-1 in HB2 cells: expression levels of both c-Myc and cyclin E, two previously identified targets for ELAVL-1 were also increased in HB2/CD151(−) cells (Fig. S8). These results suggest that CD151-dependent regulation of the ELAVL-1 function may have wider consequences for CD151-positive breast cancer cells.

ELAVL-1 is a widely expressed multifunctional RNA-binding protein that was shown to regulate alternative splicing, mRNA stability and translation by binding to AU-rich elements (AREs) in the 3′- and 5′-untranslated regions of multiple mRNAs (Lebedeva et al., 2011; Uren et al., 2011). Although specific involvement of ELAVL-1 in regulation of FGFR2 expression has not been reported previously, the *FGFR2* mRNA was identified in the transcriptome-wide screen for potential ELAVL-1 targets (Lebedeva et al., 2011). Furthermore, a recent report by Hubstenberger and colleagues demonstrated that ELAVL-1 and *FGFR2* mRNA segregate to P-bodies (Hubstenberger et al., 2017). Alternatively, ELAVL-1 can control the expression and/or alternative splicing of various proteins whose function is directly linked to the P-body assembly (Lebedeva et al., 2011; Uren et al., 2011).

RNA binding and nuclei-cytoplasm shuttling of ELAVL-1 are controlled by various post-translational modifications of the protein that involve a multitude of signalling pathways (Eberhardt et al., 2012; Grammatikakis et al., 2017). Our data suggest that CD151 regulates the activity of the protein via a PKC-dependent signalling pathway. A functional link between CD151 and cPKC has been demonstrated in two earlier studies (Li et al., 2013; Shigeta et al., 2003). Although not directly examined, both reports suggested that the expression of CD151 is correlated with the increased activity of cPKC towards their substrates. By using antibodies that detect phosphorylation of PKC substrates as our biochemical readout, we demonstrated that the activity of PKC is, in fact, suppressed in CD151-depleted cells. This poses an important question regarding

Table 2. Univariate analysis of prognostic factors

Factor	Hazard ratio	95%CI	*P* value
FGFR2	1.25	0.75-2.07	0.383
CD151	1.80	1.08-3.01	**0.023**
FGFR2(+)/CD151(−)	0.95	0.52-1.73	0.87
FGFR2(−)/CD151(+)	1.45	0.87-2.44	0.16
FGFR2(+)/CD151(+)	1.54	0.84-2.84	0.17
FGFR2(−)/CD151(−)	0.47	0.24-0.91	**0.024**
Tumour size (T1 vs T2–T4)	1.77	1.30-2.40	**<0.001**
Nodal status	3.01	1.78-5.10	**<0.001**
Stage (I vs II vs III)	1.81	1.27-2.57	**<0.001**
ER/PR	0.53	0.33-0.87	**0.011**
HER2	2.07	1.18-3.64	**0.012**
Grade (G1–2 vs G3)	1.24	0.76-2.01	0.383

Statistically significant correlation is shown in bold.

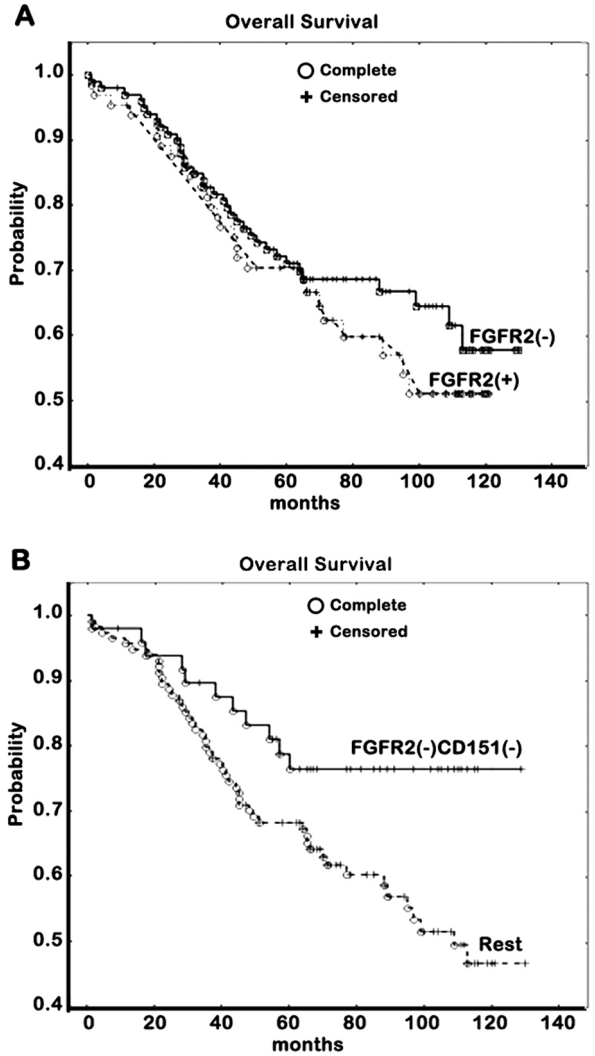


Fig. 6. Kaplan–Meier curves of overall survival for patients with breast cancer. (A) Patients expressing cytoplasmic/membranous FGFR2 compared with FGFR2-negative patients. (B) Patients negative for both FGFR2 and CD151 [FGFR2(−)CD151(−)] compared with the rest of the cohort.

the nature of the functional and physical links between cPKC and CD151 and how the removal of the tetraspanin can affect the activity of the enzymes. Previous biochemical studies have shown that the interaction between tetraspanins and cPKC occurs only under the acute cell stimulation with PMA (Gustafson-Wagner and Stipp, 2013; Zhang et al., 2001), or upon activation of EGF or B-cell receptors (Deng et al., 2012; Zuidscherwoude et al., 2017). Here, we demonstrate for the first time that a tetraspanin protein affects PKC-dependent signalling even under standard/basal growth conditions when the association between the proteins is not detected (Fig. S5). This observation indicates that a physical link between CD151 and cPKC is not necessary for the tetraspanin to modulate the activity of the enzymes towards their targets. Rather, it is likely that, functionally, CD151 (and possibly other tetraspanins) are linked to cPKC indirectly. Indeed, we show here for the first time that the removal of CD151 in breast cancer cells decreases the abundance of particular species of DAG. The importance of this observation is that it is not a general change in all DAG species, but rather specific changes in individual molecular species that is observed. We have previously discussed the differential regulation of signalling by distinct DAG species (Wakelam, 1998). Furthermore, our data indicate that the effect of CD151 on the production of DAGs (at least, some of the DAG species) may be linked with redistribution of PLCγ. Thus, we propose that CD151 affects the activity of PKC by regulating either biosynthetic or catabolic pathways linked to the generation of DAGs. Further investigation will be necessary to pinpoint how these pathways are directly targeted by the tetraspanin.

In summary, we discovered a novel mechanistic link between the CD151 complex and FGFR2. The data demonstrate that, in addition to their well-established role as post-translational regulators of protein expression, tetraspanins are also involved in regulation of protein expression at the post-transcriptional level. Future investigation into molecular pathways responsible for tetraspanin-dependent regulation of FGFR2 expression will further define the functional interdependence of tetraspanin microdomains and FGFR2 in the context of breast cancer.

MATERIALS AND METHODS

Cell lines, antibodies and reagents

HB2/CD151(+), HB2/CD151(−), HB2/α3(−), HB2/α6(−), SKBR3/CD151(+), SKBR3/CD151(−), MCF7/CD151(+) and MCF7/CD151(−) cells were described previously (Baldwin et al., 2008; Novitskaya et al., 2010, 2014). The HB2/CD151rec cell line was established after transfections of HB2/CD151(−) cells with the construct encoding the shRNA-resistant form of CD151 (Novitskaya et al., 2014). Antibodies against FGFR1 (sc-121), FGFR2 (sc-122), FGFR4 (sc-124), Src (sc-18) and TIA-1 (sc-1751) were from Santa Cruz Biotechnology. All antibodies from Santa Cruz Biotechnology were used at the dilution 1:400. Antibodies against β-actin (A5316, used at 1:10,000) were purchased from Sigma-Aldrich; all phospho-specific antibodies [phospho (Ser) PKC substrate (#2261), phospho Src family (Tyr 416) (#2113), phospho p38 (T180/Y182) (#9215) and phospho-PLCgamma1(Y783)(#2821)] and antibodies to EDC4/Ge-1 (#2548), PatL1 (#14288), TIAR (#8509), hnRNP C1/C2 (#12392) and ELAVL-1 (#12582) were from Cell Signaling Technologies. All antibodies from Cell Signaling Biotechnology were used at the dilution 1:500. All growth factors were purchased from Peprotech; laminin-rich extracellular matrix (lrECM) Matrigel, was from BD Bioscience.

Culturing of cells in 3D lrECM

Culturing cells in 3D-lrECM was performed as previously described (Sadej et al., 2009). Briefly, 1.5×10^3 cells were resuspended in 40 µl of growth factor reduced lrECM:DMEM (1:1). Solidified lrECM drops were covered with medium containing 2% fetal bovine serum (FBS) and supplemented, when needed, with FGFs (50 ng/ml). For morphological analyses in 3D culture experiments, pictures were taken using a Zeiss Primovert microscope coupled with an AxioCam ERc 5s camera (Zeiss). Cell proliferation in 3D was measured using WST-1 reagent (tetrazolium salts) according to the manufacturer's protocol (Roche). Experiments were performed in duplicate, and at least three independent experiments were performed for each experimental condition. Comparative data were analysed with an unpaired Student's *t*-test using the Statistica 7.1 software. Differences for which $P<0.05$ were considered as statistically significant.

Western blotting

Cells grown to 80–90% confluence were lysed in Laemmli buffer supplemented with 2 mM PMSF, 10 µg/ml aprotinin, 10 µg/ml leupeptin, 5 mM EGTA, 1 mM EDTA, 2 mM $Na_4P_2O_7$, 5 mM NaF and 5 mM Na_3VO_4. Samples containing equal amounts of protein per lane were loaded and resolved on 10% SDS-PAGE and then transferred onto nitrocellulose membrane. The membranes were incubated for 1 h in 5% skimmed milk and probed with specific antibodies overnight. The infrared-tagged secondary antibodies were used to visualize the signals, and the images were captured and quantified using a LI-COR Odyssey scanning system.

siRNA-based knockdown

Cells were transfected with control or gene-targeting siRNAs (20 nM) using Lipofectamine®RNAiMAX transfection reagent (ThermoFisher). The expression level of FGFR2 was assessed by western blotting at 72 h after transfection. For immunofluorescence experiments, cells were plated on coverslips the day before transfection and analysed 72 h after transfection. All siRNAs were purchased from Qiagen in the Flexitube format; target sequences are shown in Table S1.

Immunofluorescence staining

Cells grown on glass coverslips were fixed with 4% paraformaldehyde and permeabilized using 0.1% Triton X-100. Staining with rabbit anti-EDC4 antibody (diluted at 1:200) was carried out according to the manufacturer's recommendations. P-bodies were quantified in 50–100 cells in two or three independent experiments. For staining using anti-PLCγ1 antibody (diluted at 1:100, Cell Signaling Technology, #2282) cells were permeabilized for 1 h in 1%Brij98.

Real-time qPCR

Total RNA was isolated from HB2 cells using the RNAeasy kit according to the manufacturer's instructions (Qiagen, Crawley, United Kingdom). The cDNAs were synthesized from RNA (1 µg) by MultiScribe™ MuLV reverse transcriptase (Life Technology, Paisley, UK). Real-time quantitative (q)PCR was carried out using either commercial TaqMan or custom-designed SYBR Green primers (sequences are available upon request). PCR conditions were as follows: (1) 95°C for 10 min; (2) 40 cycles of 95°C for 15 s and 60°C for 1 min. At least three separate PCR experiments for each gene were performed. The real-time amplification data were analysed using REST software (Qiagen, Crawley, UK) and gene expression levels were normalized relative to that of the control *GAPDH* gene for the TaqMan assay or β-actin for the SYBR green assay.

Diacylglycerol analysis by mass spectrometry

Frozen cell pellet lipids were extracted using the Folch method with chloroform:methanol:water (2:1:1 ratio) and resuspended in chloroform:methanol (1:1) prior to injection into a Shimadzu Prominence 20-AD system (Shimadzu, Kyoto, Japan). Chromatographic separation was achieved upon a Waters Acquity UPLC C_4 (100×1 mm, 1.7 µm particle size) column (Milford, MA). The column was kept at 45°C, and 7 µl of samples were eluted using a mobile phase composed of solvent A (water) and B (acetronitile) each containing 0.025% formic acid. The gradient started at 45% B (5 min), with an increase to 90% B for 5 min; 100% B was reached after an additional 10 min and held for 7 min before re-equilibration at 45% B for 5 min. The flow rate was maintained constant at 100 µl/min. Accurate mass (with an error below 5 ppm) was acquired on an Orbitrap Elite mass spectrometer (Thermo Fisher Scientific, Waltham, MA). Source parameters for positive polarity were: capillary temperature, 275°C; source heater

temperature, 200°C; sheath gas, 10 AU; aux gas, 5 AU; and sweep gas, 5 AU. The source voltage was 3.8 kV. Full scan spectra in the range of *m/z* 150 to 1000 were acquired at a target resolution of 240,000 [full width at half maximum (FWHM) at *m/z* 400]. Data analysis was performed using Lipid Data Analyzer (2.6.0_2) software (Hartler et al., 2017).

Patient selection and samples

Patient selection and samples are described in Table S2. Specimens of primary invasive ductal carcinoma from women treated in the Oncology Department of the Copernicus Memorial Hospital in Lodz, Poland between 2003 and 2010 were obtained according to the local ethical regulations (License No. RNN/174/11KE, Medical University of Lodz 2011) and according to the principles expressed in the Declaration of Helsinki (2000). This is archival material and no consent specific for this study was required.

Immunohistochemistry

Serial 5 µm paraffin sections of archival formalin-fixed blocks were processed for immunohistochemistry for FGFR2 (rabbit anti-human; 1:100; Santa Cruz Biotechnology) and CD151 (mouse anti-human; 1:100; Novocastra, UK) using routine protocols described previously (Novitskaya et al., 2014). As a negative control for immunostaining, primary antibodies were replaced by nonimmune sera. Scoring immunoreactivity for FGFR2 was carried out separately for cytoplasmic/membranous and nuclear FGFR2 expression. Cytoplasmic/membranous expression of FGFR2 was considered: (1) 0/negative, if no reactivity, (2) 1+/positive, if weak to moderate membranous and/or cytoplasmic staining was observed in <10% of tumour cells; (3) 2+/positive, if moderate membranous and/or cytoplasmic staining was observed in ≥10% of tumour cells; and (4) 3+/positive, if strong membranous and/or cytoplasmic staining was observed in ≥10% of the tumour cells. Assessment of nuclear immunoreactivity was based on Quick score (Detre et al., 1995). Scoring of immunoreactivity for CD151 was performed as described previously (Novitskaya et al., 2014). Immunohistochemical staining was assessed independently by two observers (H.R.-K. and R.K.) who were blind to the clinicopathological data.

Statistical analysis

Overall survival was calculated from the date of surgery to the date of death or the last follow-up, as recommended by the Kaplan–Meier method. Differences in survival distributions were compared using a log-rank test. Data for patients who died from other causes than breast cancer were censored at the time of death. Univariate and multivariate analyses of overall survival were performed using the Cox's proportional hazards regression model. Pearson's test or Fisher's exact test were used to assess the associations between expression of FGFR2 and CD151 alone and their co-expression and clinicopathological variables. Kendall's tau rank correlation test was used to study correlation between levels of FGFR2 and CD151 expression in cancer tissue. The results were considered statistically significant when a two-sided test gave $P<0.05$. The analyses were performed using the StatsDirect (StatsDirect Ltd, Altrincham, UK) and Statistica 9.1 (StatSoft Inc., Tulsa, OK, USA) software.

Acknowledgements

We would like to thank Dr Renata Kusińska for making available tissue samples from the patients with IDC.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: M.J.W., F.B.; Methodology: M.J.W., F.B.; Formal analysis: R.S., X.L., V.N., R.K., P.P., M.J.W., H.R., F.B.; Data curation: X.L., L.T., V.N., A.F.L., H.R.-K.; Writing - original draft: R.S., P.P., H.R., F.B.; Writing - review & editing: A.F.L., M.J.W., H.R., F.B.; Supervision: F.B.; Funding acquisition: H.R., F.B.

Funding

This work was supported by Narodowym Centrum Nauki (NCN) grants #2013/B/NZ4/02512 (to H.R.-K.) and #2012/06/M/NZ3/00023 (to R.S.), Fundacja na rzecz Nauki Polskiej (Foundation for Polish Science) (HOMING PLUS/2010-2/12) (to R.S.), Breast Cancer Campaign grant 2012PR113 (to F.B.) and the Biotechnology and Biological Sciences Research Council (BBSRC) #BB/P013384/1 (to M.J.O.W.).

Supplementary information

Supplementary information available online at
http://jcs.biologists.org/lookup/doi/10.1242/jcs.220640.supplemental

References

Anderson, P., Kedersha, N. and Ivanov, P. (2015). Stress granules, P-bodies and cancer. *Biochim. Biophys. Acta* **1849**, 861-870.

Bai, A., Meetze, K., Vo, N. Y., Kollipara, S., Mazsa, E. K., Winston, W. M., Weiler, S., Poling, L. L., Chen, T., Ismail, N. S. et al. (2010). GP369, an FGFR2-IIIb-specific antibody, exhibits potent antitumor activity against human cancers driven by activated FGFR2 signaling. *Cancer Res.* **70**, 7630-7639.

Baldwin, G., Novitskaya, V., Sadej, R., Pochec, E., Litynska, A., Hartmann, C., Williams, J., Ashman, L., Eble, J. A. and Berditchevski, F. (2008). Tetraspanin cd151 regulates glycosylation of alpha3beta1 integrin. *J. Biol. Chem.* **283**, 35445-35454.

Berditchevski, F. and Rubinstein, E. (2013). *Tetraspanins*. Springer.

Buchan, J. R. (2014). mRNP granules. Assembly, function, and connections with disease. *RNA Biol.* **11**, 1019-1030.

Cox, D. G., Curtit, E., Romieu, G., Fumoleau, P., Rios, M., Bonnefoi, H., Bachelot, T., Soulie, P., Jouannaud, C., Bourgeois, H. et al. (2016). GWAS in the SIGNAL/PHARE clinical cohort restricts the association between the FGFR2 locus and estrogen receptor status to HER2-negative breast cancer patients. *Oncotarget* **7**, 77358-77364.

Cui, F., Wu, D., Wang, W., He, X. and Wang, M. (2016). Variants of FGFR2 and their associations with breast cancer risk: a HUGE systematic review and meta-analysis. *Breast Cancer Res. Treat.* **155**, 313-335.

Czaplinska, D., Mieczkowski, K., Supernat, A., Skladanowski, A. C., Kordek, R., Biernat, W., Zaczek, A. J., Romanska, H. M. and Sadej, R. (2016). Interactions between FGFR2 and RSK2-implications for breast cancer prognosis. *Tumour. Biol.* **37**, 13721-13731.

Del Gatto-Konczak, F., Bourgeois, C. F., Le Guiner, C., Kister, L., Gesnel, M. C., Stévenin, J. and Breathnach, R. (2000). The RNA-binding protein TIA-1 is a novel mammalian splicing regulator acting through intron sequences adjacent to a 5' splice site. *Mol. Cell Biol.* **20**, 6287-6299.

Deng, X., Li, Q., Hoff, J., Novak, M., Yang, H., Jin, H., Erfani, S. F., Sharma, C., Zhou, P., Rabinovitz, I. et al. (2012). Integrin-associated CD151 drives ErbB2-evoked mammary tumor onset and metastasis. *Neoplasia* **14**, 678-689.

Detre, S., Saclani Jotti, G. and Dowsett, M. (1995). A "quickscore" method for immunohistochemical semiquantitation: validation for oestrogen receptor in breast carcinomas. *J. Clin. Pathol.* **48**, 876-878.

Eberhardt, W., Doller, A. and Pfeilschifter, J. (2012). Regulation of the mRNA-binding protein HuR by posttranslational modification: spotlight on phosphorylation. *Curr. Protein Pept. Sci.* **13**, 380-390.

Fearon, A. E., Gould, C. R. and Grose, R. P. (2013). FGFR signalling in women's cancers. *Int. J. Biochem. Cell Biol.* **45**, 2832-2842.

Grammatikakis, I., Abdelmohsen, K. and Gorospe, M. (2017). Posttranslational control of HuR function. *Wiley. Interdiscip. Rev. RNA* **8**, e1372.

Gustafson-Wagner, E. and Stipp, C. S. (2013). The CD9/CD81 tetraspanin complex and tetraspanin CD151 regulate alpha3beta1 integrin-dependent tumor cell behaviors by overlapping but distinct mechanisms. *PLoS ONE* **8**, e61834.

Hartler, J., Triebl, A., Ziegl, A., Trötzmüller, M., Rechberger, G. N., Zeleznik, O. A., Zierler, K. A., Torta, F., Cazenave-Gassiot, A., Wenk, M. R. et al. (2017). Deciphering lipid structures based on platform-independent decision rules. *Nat. Methods* **14**, 1171-1174.

Hasegawa, M., Furuya, M., Kasuya, Y., Nishiyama, M., Sugiura, T., Nikaido, T., Momota, Y., Ichinose, M. and Kimura, S. (2007). CD151 dynamics in carcinoma-stroma interaction: integrin expression, adhesion strength and proteolytic activity. *Lab. Invest.* **87**, 882-892.

Hemler, M. E. (2014). Tetraspanin proteins promote multiple cancer stages. *Nat. Rev. Cancer* **14**, 49-60.

Hubstenberger, A., Courel, M., Bénard, M., Souquere, S., Ernoult-Lange, M., Chouaib, R., Yi, Z., Morlot, J.-B., Munier, A., Fradet, M. et al. (2017). P-body purification reveals the condensation of repressed mRNA regulons. *Mol. Cell* **68**, 144-157.e5.

Izquierdo, J. M. (2010). Heterogeneous ribonucleoprotein C displays a repressor activity mediated by T-cell intracellular antigen-1-related/like protein to modulate Fas exon 6 splicing through a mechanism involving Hu antigen R. *Nucleic Acids Res.* **38**, 8001-8014.

Johnson, J. L., Winterwood, N., DeMali, K. A. and Stipp, C. S. (2009). Tetraspanin CD151 regulates RhoA activation and the dynamic stability of carcinoma cell-cell contacts. *J. Cell Sci.* **122**, 2263-2273.

Katoh, M. (2008). Cancer genomics and genetics of FGFR2 (Review). *Int. J. Oncol.* **33**, 233-237.

Katoh, M. (2009). FGFR2 abnormalities underlie a spectrum of bone, skin, and cancer pathologies. *J. Invest Dermatol.* **129**, 1861-1867.

Katoh, M. and Nakagama, H. (2014). FGF receptors: cancer biology and therapeutics. *Med. Res. Rev.* **34**, 280-300.
Kelleher, F. C., O'Sullivan, H., Smyth, E., McDermott, R. and Viterbo, A. (2013). Fibroblast growth factor receptors, developmental corruption and malignant disease. *Carcinogenesis* **34**, 2198-2205.
Klosek, S. K., Nakashiro, K., Hara, S., Goda, H., Hasegawa, H. and Hamakawa, H. (2009). CD151 regulates HGF-stimulated morphogenesis of human breast cancer cells. *Biochem. Biophys. Res. Commun.* **379**, 1097-1100.
Kwon, M. J., Park, S., Choi, J. Y., Oh, E., Kim, Y. J., Park, Y.-H., Cho, E. Y., Kwon, M. J., Nam, S. J., Im, Y.-H. et al. (2012). Clinical significance of CD151 overexpression in subtypes of invasive breast cancer. *Br. J. Cancer* **106**, 923-930.
Lebedeva, S., Jens, M., Theil, K., Schwanhäusser, B., Selbach, M., Landthaler, M. and Rajewsky, N. (2011). Transcriptome-wide analysis of regulatory interactions of the RNA-binding protein HuR. *Mol. Cell* **43**, 340-352.
Li, Q., Yang, X. H., Xu, F., Sharma, C., Wang, H.-X., Knoblich, K., Rabinovitz, I., Granter, S. R. and Hemler, M. E. (2013). Tetraspanin CD151 plays a key role in skin squamous cell carcinoma. *Oncogene* **32**, 1772-1783.
Novitskaya, V., Romanska, H., Dawoud, M., Jones, J. L. and Berditchevski, F. (2010). Tetraspanin CD151 regulates growth of mammary epithelial cells in three-dimensional extracellular matrix: implication for mammary ductal carcinoma in situ. *Cancer Res.* **70**, 4698-4708.
Novitskaya, V., Romanska, H., Kordek, R., Potemski, P., Kusińska, R., Parsons, M., Odintsova, E. and Berditchevski, F. (2014). Integrin alpha3beta1-CD151 complex regulates dimerization of ErbB2 via RhoA. *Oncogene* **33**, 2779-2789.
Ozgur, S., Chekulaeva, M. and Stoecklin, G. (2010). Human Pat1b connects deadenylation with mRNA decapping and controls the assembly of processing bodies. *Mol. Cell Biol.* **30**, 4308-4823. Epub 2010 Jun 28.
Robbez-Masson, L. J., Bödör, C., Jones, J. L., Hurst, H. C., Fitzgibbon, J., Hart, I. R. and Grose, R. P. (2013). Functional analysis of a breast cancer-associated FGFR2 single nucleotide polymorphism using zinc finger mediated genome editing. *PLoS ONE* **8**, e78839.
Sadej, R., Romanska, H., Baldwin, G., Gkirtzimanaki, K., Novitskaya, V., Filer, A. D., Krcova, Z., Kusinska, R., Ehrmann, J., Buckley, C. D. et al. (2009). CD151 regulates tumorigenesis by modulating the communication between tumor cells and endothelium. *Mol. Cancer Res.* **7**, 787-798.
Sadej, R., Romanska, H., Kavanagh, D., Baldwin, G., Takahashi, T., Kalia, N. and Berditchevski, F. (2010). Tetraspanin CD151 regulates transforming growth factor beta signaling: implication in tumor metastasis. *Cancer Res.* **70**, 6059-6070.
Sadej, R., Grudowska, A., Turczyk, L., Kordek, R. and Romanska, H. M. (2014). CD151 in cancer progression and metastasis: a complex scenario. *Lab. Invest.* **94**, 41-51.
Shigeta, M., Sanzen, N., Ozawa, M., Gu, J., Hasegawa, H. and Sekiguchi, K. (2003). CD151 regulates epithelial cell-cell adhesion through PKC- and Cdc42-dependent actin cytoskeletal reorganization. *J. Cell Biol.* **163**, 165-176.
Sommer, A., Kopitz, C., Schatz, C. A., Nising, C. F., Mahlert, C., Lerchen, H.-G., Stelte-Ludwig, B., Hammer, S., Greven, S., Schuhmacher, J. et al. (2016). Preclinical efficacy of the auristatin-based antibody-drug conjugate BAY 1187982 for the treatment of FGFR2-positive solid tumors. *Cancer Res.* **76**, 6331-6339.
Stalder, L. and Mühlemann, O. (2009). Processing bodies are not required for mammalian nonsense-mediated mRNA decay. *RNA* **15**, 1265-1273.
Stoecklin, G. and Kedersha, N. (2013). Relationship of GW/P-bodies with stress granules. *Adv. Exp. Med. Biol.* **768**, 197-211.
Tarkkonen, K. M., Nilsson, E. M., Kähkönen, T. E., Dey, J. H., Heikkilä, J. E., Tuomela, J. M., Liu, Q., Hynes, N. E. and Härkönen, P. L. (2012). Differential roles of fibroblast growth factor receptors (FGFR) 1, 2 and 3 in the regulation of S115 breast cancer cell growth. *PLoS ONE* **7**, e49970.
Termini, C. M. and Gillette, J. M. (2017). Tetraspanins function as regulators of cellular signaling. *Front. Cell Dev. Biol.* **5**, 34.
Turner, N., Lambros, M. B., Horlings, H. M., Pearson, A., Sharpe, R., Natrajan, R., Geyer, F. C., van Kouwenhove, M., Kreike, B., Mackay, A. et al. (2010). Integrative molecular profiling of triple negative breast cancers identifies amplicon drivers and potential therapeutic targets. *Oncogene* **29**, 2013-2023.
Uren, P. J., Burns, S. C., Ruan, J., Singh, K. K., Smith, A. D. and Penalva, L. O. F. (2011). Genomic analyses of the RNA-binding protein Hu antigen R (HuR) identify a complex network of target genes and novel characteristics of its binding sites. *J. Biol. Chem.* **286**, 37063-37066.
Wakelam, M. J. O. (1998). Diacylglycerol–when is it an intracellular messenger? *Biochim. Biophys. Acta* **1436**, 117-126.
Wu, Y.-M., Su, F., Kalyana-Sundaram, S., Khazanov, N., Ateeq, B., Cao, X., Lonigro, R. J., Vats, P., Wang, R., Lin, S.-F. et al. (2013). Identification of targetable FGFR gene fusions in diverse cancers. *Cancer Discov.* **3**, 636-647.
Xian, W., Schwertfeger, K. L. and Rosen, J. M. (2007). Distinct roles of fibroblast growth factor receptor 1 and 2 in regulating cell survival and epithelial-mesenchymal transition. *Mol. Endocrinol.* **21**, 987-1000.
Zhang, X. A., Bontrager, A. L. and Hemler, M. E. (2001). Transmembrane-4 superfamily proteins associate with activated protein kinase C (PKC) and link PKC to specific beta1 integrins. *J. Biol. Chem.* **276**, 25005-22513.
Zuidscherwoude, M., Dunlock, V.-M. E., van den Bogaart, G., van Deventer, S. J., van der Schaaf, A., van Oostrum, J., Goedhart, J., In 't Hout, J., Hammerling, G. J., Tanaka, S. et al. (2017). Tetraspanin microdomains control localized protein kinase C signaling in B cells. *Sci. Signal.* **10**, eaag2755.