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Characterization of the fibroblast growth factor receptor 2 splicing mechanism in triple-negative breast cancer

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ABSTRACT

Breast cancer is the second most frequently diagnosed cancer in the world and the most frequently diagnosed cancer in women. Triple-negative breast cancer (TNBC), a subtype of breast cancer, is characterised by the lack of expression of the oestrogen receptor, progesterone receptor and a lack of overexpression of the human epidermal growth factor receptor 2. Therapeutic options targeting TNBCs are limited, and since it can be more aggressive compared to other breast cancer subtypes, research into its progression is necessary. A number of genome-wide association studies have identified an association between the fibroblast growth factor receptor (FGFR) 2 and the risk of breast cancer. This receptor is alternatively spliced, and a switch in the mutually exclusive inclusion of either the *FGFR2 IIIb* or the *FGFR2 IIIc* exons is associated with epithelial-to-mesenchymal transition (EMT) and mesenchymal-to-epithelial transition (MET). A nuclear proteomic analysis comparing an epithelial TNBC cell line to a mesenchymal one revealed that the RNA binding protein, KH-type Splicing Regulatory Protein (KSRP), is overexpressed in the basal cell line compared to the mesenchymal cell line; these cell lines preferentially include *FGFR2 IIIb* and *FGFR2 IIIc*, respectively.

This project aimed to investigate the role of KSRP in the *FGFR2* splicing mechanism in the context of triple-negative breast cancer progression using epithelial and mesenchymal cell line models. In addition, this project aimed to discover the genome-wide consequences of KSRP knockdown.

The epithelial and mesenchymal phenotypes, preferential *FGFR2* isoform inclusion, differential expression of KSRP and alteration of *FGFR2* isoform expression in MDA-MB-468 (epithelial) and MDA-MB-436 (mesenchymal) cells were confirmed using fluorescent microscopy, semi-quantitative polymerase chain reaction, quantitative real-time PCR (qRT-PCR), and western blotting. qRT-PCR was used to determine whether the expression of *FGFR2 IIIb* and *FGFR2 IIIc* became altered in response to changes in the expression of KSRP (either up- or downregulation). Fluorescent microscopy was used to determine if changes in the phenotypes of the epithelial and mesenchymal cells were the consequence of the KSRP mediated changes in *FGFR2 IIIb* and *FGFR2 IIIc* expression. The

ultraviolet cross-linking and immunoprecipitation (UV-CLIP) technique was used to investigate whether KSRP binds directly to *FGFR2* mRNA in epithelial cells. We report that the role of KSRP in *FGFR2* splicing involves the tissue-specific control of the expression of *FGFR2 IIIb* and *FGFR2 IIIc*. KSRP up- and downregulation in MDA-MB-436 causes an upregulation of *FGFR2 IIIb* and *FGFR2 IIIc* expression. KSRP up- and downregulation in MDA-MB-468 cells causes a downregulation of *FGFR2 IIIb* and *FGFR2 IIIc* expression. The efficiency of the upregulation (in MDA-MB-436 cells) and downregulation (in MDA-MB-468 cells) in *FGFR2 IIIb* expression is different to that of *FGFR2 IIIc* expression, this is dependent on the level of KSRP expression. A clear switch from the splicing of *FGFR2 IIIb* to *FGFR2 IIIc*, or vice versa, in response to manipulation of KSRP expression, was not observed in epithelial and mesenchymal cells, indicating that this protein does not control the mutually exclusive splicing nature of this gene. We also show that KSRP does not control EMT or MET in epithelial or mesenchymal cells and that KSRP directly binds to *FGFR2* mRNA at exon 9 (*FGFR2 IIIb*) in epithelial cells. This role of KSRP in *FGFR2* isoform inclusion and the direct binding of KSRP to *FGFR2* mRNA have not been previously reported.

The Affymetrix GeneChip® Human Transcriptome Array 2.0 was used to identify the genome-wide consequences of KSRP knockdown in epithelial cells. Numerous significant changes in gene expression in response to KSRP knockdown were observed. In particular, three genes involved in the canonical Wnt pathway – namely *Cyclin D3* (*CCND3)*, *Dickkopf WNT Signaling Pathway Inhibitor 1* (*DKK1)* and *Protein Phosphatase 2, Regulatory Subunit A, Beta (PPP2R1B)* – were upregulated in response to KSRP downregulation, suggesting a regulatory role of KSRP in this pathway. *CCND3*, *DKK1* and *PPP2R1B* were chosen for further analysis and the change in their expression in response to KSRP downregulation was validated using qRT-PCR. A possible direct interaction between KSRP and the selected targets was predicted using STRING and investigated using confocal fluorescence microscopy. KSRP was not predicted to interact with any of these proteins but interestingly co-localises with DKK1. This suggests that KSRP and DKK1 directly interact and that KSRP could antagonise Wnt signalling. This link between KSRP, DKK1 and the Wnt pathway has not been previously reported.