SHORT REPORT

Coexpression of an unusual form of the *EWS-WT1* fusion transcript and interleukin 2/15 receptor β mRNA in a desmoplastic small round cell tumour

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Background: The β chain of the interleukin 2/15 receptor (IL-2/15R β) is induced by the expression of the *EWS-WT1*. A case of desmoplastic small round cell tumour (DSRCT) expressing only an unusual *EWS-WT1* treated by us is reported here.

Aim: To characterise an unusual form of *EWS-WT1*.

Methods: Frozen tissue sections of the axillary tumour were examined using a laser-assisted microdissection technique and reverse transcriptase polymerase chain reaction.

Results: The novel fusion of exon 8 of *EWS* and the defective exon 10 of *WT1* (–*KTS*) was detected. Although it was an unusual form, the coexpression of the present *EWS-WT1*, *IL-2/15R β* and Janus kinase (*JAK1*) mRNA was detected in the tumour cells. *IL-2* and signal transducers and activators of transcription (*STAT5*) mRNA were detected in both tumour and stromal cells.

Conclusion: The induction of the IL-2/15 receptor signalling pathway may contribute to tumorigenesis in DSRCT through a paracrine or an autocrine system, even though the *EWS-WT1* was an unusual form.

Desmoplastic small round cell tumour (DSRCT) is a rare malignant tumour, described by Gerald and Rosai in 1989,¹ found mainly in young male adolescents. Typically, the tumour presents with abdominal serosal involvement, poor prognosis and a primitive histological appearance.^{1,2} Genetic analysis showed a specific translocation in the Ewing's sarcoma gene (*EWS*) and the Wilms's tumour gene (*WT1*), which produces a chimeric transcriptional message.^{3–4} An in-frame junction of *EWS* exon 7 to *WT1* exon 8 has been detected in most cases of DSRCT. A comprehensive genetic analysis showed that interleukin 2/15 receptor β chain (IL-2/15R β) was highly expressed in cells expressing *EWS-WT1* and suggested that the IL-2/15R signalling pathway contributed to tumorigenesis in DSRCT.⁵ We treated a rare DSRCT case in which the tumour cells expressed only unusual *EWS-WT1*. Therefore, our study aimed at evaluating the expression of IL-2/15R β and its downstream mRNA and correlating it with the present *EWS-WT1*.

METHODS

Patient

The patient was a 25-year-old man. A nodular lesion was identified in his left lung by a chest radiograph in 1997. In 2001, a thoroscopic examination showed multiple nodules in his left lung. Axillary, skin and head metastases were also identified. The patient refused to undergo chemotherapy. He

was followed up in the outpatient clinic, but he died in 2003. His left lung and axillary tumour were solid and well circumscribed, measuring 1.5 cm $\times$ 1.5 cm and 4.0 cm $\times$ 2.6 cm in size, respectively. Histopathologically, the tumour consisted of solid sheets, nests or small clumps of round or spindle cells with clear cytoplasm and intermingled rhabdoid cells, embedded in desmoplastic stroma (fig 1A). Immunohistochemically, the tumour cells were focally positive only for wide-molecule cytokeratin, but were negative for high-molecule and low-molecule cytokeratins. Calretinin, neurone-specific enolase, WT1 and vimentin were positive. Desmin, S-100 protein and CD34 were negative.

RT-PCR

Frozen tissue sections, 8 μ m in thickness, were obtained from the axillary tumour. Tumour and stromal cells were collected separately by a laser-assisted microdissection technique.⁶ Total RNA was extracted and reverse transcribed as described previously.⁶ PCR assays were carried out for 50 cycles at 95°C for 30 s, 60°C for 1 min and 72°C for 1 min with the primer pairs listed in table 1. RT-PCR products were electrophoresed on a 2% agarose gel and stained with SYBR Green I (Cambrex Bio Science, Rockland, Maine, USA). Each amplified fragment of the *EWS-WT1* was purified and sequencing analysis was carried out by Prism 310 genetic analyzer (Applied Biosystems Japan, Tokyo, Japan).

RESULTS

In the present case, the novel *EWS-WT1* fusion transcript of *EWS* exon 8 and *WT1* exon 10 without the sequence coding a Lys-Thr-Ser (–*KTS*) was identified, but the typical *EWS-WT1* fusion of *EWS* exon 7 and *WT1* exon 8 or another form of *EWS-WT1* was not detected (fig 1B,C). In addition, a 6-bp deletion at the 5' end of *WT1* exon 10 and a 1-bp insertion in the junction of *EWS-WT1* were present (fig 1C). An RT-PCR assay with the conventional primer pair of *EWS* exon 7 and *WT1* exon 8 amplified only the typical *EWS-WT1* in the control DSRCT (fig 1D, grey arrow), but not any other *EWS-WT1* in the present case. The present *EWS-WT1* was amplified with the primer pair of *EWS* exon 8 and *WT1* exon 10 (fig 1D, white arrows). In whole-tissue sections of both typical and the present DSRCT, RT-PCR analysis showed the coexpression of the *EWS-WT1*, *IL-2*, *IL-2/15R β* , *JAK1* and *STAT5* mRNA. Furthermore, we investigated the difference in mRNA expressions between the tumour cells and the stromal cells. Although the *EWS-WT1*, *IL-2/15R β* and *JAK1* mRNA

Abbreviations: DSRCT, desmoplastic small round cell tumour; *EWS*, Ewing's sarcoma gene; *IL-2/15R β* , interleukin 2/15 receptor β chain; *JAK*, Janus kinase; RT-PCR, reverse transcriptase polymerase chain reaction; *STAT5*, signal transducers and activators of transcription; *WT1*, Wilms's tumour gene

Figure 1 (A) Microscopic image of a section stained with haematoxylin and eosin ($\times 200$). (B) Single fragment amplified by reverse transcriptase polymerase chain reaction (RT-PCR) assay with the conventional primer pair of Ewing's sarcoma gene (*EWS*) exon 7 and Wilms's tumour gene (*WT1*) exon 10 (primer A). The DNA ladder marker (left lane) and the present desmoplastic small round cell tumour (DSRCT) sample (right lane) are shown. (C) Sequencing analysis showed that the present cDNA was composed of the fusion of exon 8 of *EWS* and exon 10 of *WT1*, and contained a 6-bp deletion at the 5' end of *WT1* exon 10, a 1-bp insertion and no sequence coding a Lys-Thr-Ser (KTS) in the junction. This aberrant sequence was also identified by the sequencing analysis with primer B. The combined sequence of inserted "c" and *WT1* exon 10 was not homologous with any intronic sequences. (D) RT-PCR with the conventional primer pair for *EWS* exon 7 and *WT1* exon 8 (primer C) amplified only the typical *EWS*-*WT1* in the control DSRCT (grey arrow). The present *EWS*-*WT1* was amplified with primer B (white arrow). The lower bands in the *EWS*-*WT1* gel for the whole tumour and the dissected tumour of the patient were not confirmed as a specific sequence. RT-PCR assays showed the coexpression of the *EWS*-*WT1*, *IL-2*, *IL-2/15R β* , Janus kinase (*JAK1*) and signal transducers and activators of transcription (*STAT5*) mRNA. Furthermore, the present *EWS*-*WT1*, *IL-2/15R β* and *JAK1* mRNA were detected only in tumour cells, and *IL-2* and *STAT5* mRNA were detected in both tumour and stromal cells. β actin mRNA was detected as an internal control.

were detected only in tumour cells, *IL-2* and *STAT5* mRNA were detected in both tumour and stromal cells (fig 1D).

DISCUSSION

The *EWS*-*WT1* fusion transcripts, typically at the in-frame junction of *EWS* exon 7 and *WT1* exon 8, have been detected

by RT-PCR in most documented cases of DSRCT.⁷ Molecular variants have been reported.^{8,9} Although Liu *et al*⁹ have reported the fusion of *EWS* exons 1-5 and *WT1* exon 10, this tumour also expressed the typical *EWS*-*WT1*.⁹ The present tumour, however, expressed only an unusual transcript between *EWS* exon 8 and the defective *WT1* exon 10 (-KTS). RT-PCR assays with the conventional primer pairs

Table 1 DNA oligonucleotides used in RT-PCR sequencing

Gene/exon	Sense/antisense	References	Sequence	Product size (bp)
Primer A				
<i>EWS</i> /exon 7	Sense	Ladanyi and Gerald ³	5'-tctacagccaagctccaagtc-3'	
<i>WT1</i> /exon 10	Antisense	Ladanyi and Gerald ³	5'-gccaccagcagctgaagggc-3'	
Primer B				
<i>EWS</i> /exon 8	Sense		5'-catgagtgccctgataacc-3'	
<i>WT1</i> /exon 10	Antisense		5'-accgggcaaacctttctg-3'	
Primer C				
<i>EWS</i> /exon 7	Sense	Ladanyi and Gerald ³	5'-tctacagccaagctccaagtc-3'	
<i>WT1</i> /exon 8	Antisense	Gerald <i>et al</i> ²	5'-acctcgttcacagtcctt-3'	
<i>IL-2</i>	Sense		5'-aactcaccagatgctaca-3'	
	Antisense		5'-gcacttctccagaggttg-3'	105
<i>IL-2/15Rβ</i>	Sense		5'-aatggcactccagttcac-3'	
	Antisense		5'-agcagctcacaggttggtt-3'	146
<i>JAK1</i>	Sense		5'-ggcgctattctccaagaag-3'	
	Antisense		5'-aataggagccaggcatttca-3'	140
<i>STAT5</i>	Sense		5'-gtcctgaagaccagaccac-3'	
	Antisense		5'-gttgccgggtgttctcattt-3'	150
β -actin	Sense		5'-aaactggaacgggtgaaggtg-3'	
	Antisense		5'-gtggcttttaggatggcaag-3'	160

EWS, Ewing's sarcoma gene; *JAK1*, Janus kinase; *STAT5*, signal transducers and activators of transcription; *WT1*, Wilms's tumour gene.

Take-home messages

- In the desmoplastic small round cell tumour (DSRCT), novel fusion of only exon 8 of the Ewing's sarcoma gene (*EWS*) with the defective exon 10 of the Wilms' tumour gene (*WT1*) (–*KTS*) was detected.
- Coexpression of the unusual *EWS–WT1*, interleukin 2/15 receptor β (*IL-2/15R β*) and *JAK1* mRNA were detected in the tumour cells. *IL-2* and *STAT5* mRNA were detected in both tumour and stromal cells.
- Coexpression of *EWS–WT1*, *IL-2/15R β* and its downstream mRNA may suggest that the induction of the *IL-2/15* receptor signalling pathway may contribute to tumorigenesis in DSRCT through a paracrine or an autocrine system, even though the *EWS–WT1* was an unusual form.

did not amplify any other *EWS–WT1*, although the typical fusion was detectable in another case. Further investigation needs to clarify whether these results occurred by the transcription error or by unusual genomic fusion. Transcriptional targets of *EWS–WT1* have been analysed.^{5 10 11} Wong *et al*⁵ have reported that the *IL-2/15R β* transcript was induced by *EWS–WT1* directly, but not by wild-type *WT1* and *EWS–WT1* including a Lys-Thr-Ser (+*KTS*), an alternative splice form of *WT1*⁵ with three amino acids (*KTS*) between zinc fingers 3 and 4.¹² The fusion of *EWS* exon 8 and *WT1* exon 8 is frame shifted by *EWS* exon 8 and did not bind to the target.¹⁰ One reported case of DSRCT expressed the fusion of *EWS* exon 8 and *WT1* exon 8, but it contained a 4-bp deletion and a 6-bp insertion. By this mutation, the reading frame of *WT1* may have been incidentally preserved. The present tumour must have further gene mutations in *EWS* exons 1–8 to have preserved the reading frame of *WT1* exon 10. RT-PCR analysis, however, showed the simultaneous expression of *IL-2/15 R β* signalling mRNA and *EWS–WT1*. We may suggest that the present *EWS–WT1* had the effect of transcriptional activity, although its form was incomprehensible. In addition, the *EWS–WT1* and *IL-2/15R β* mRNA were coexpressed only in tumour cells and *IL-2* mRNA was expressed in both tumour and stroma cells. *IL-2* expression was not detected in DSRCT tumour cells themselves.⁵ *IL-2* message may, however, be produced also by DSRCT tumour cells themselves.

In this study, the actual transcriptional activity by the present *EWS–WT1* was not clarified. Coexpression of the present *EWS–WT1*, *IL-2/15R β* and its downstream mRNA,

however, may suggest that the induction of the *IL-2/15R* signalling pathway may contribute to tumorigenesis in DSRCT through a paracrine or an autocrine system, even though the *EWS–WT1* was an unusual form.

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