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Differential expression of neural markers in KIT and PDGFRA wild-type gastrointestinal stromal tumours

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Aims: To compare the genomic signatures of wild-type (WT) and mutated GISTs and the murine interstitial cells of Cajal (ICCs) to find markers of cell differentiation and other functions that may identify cells that give rise to WT tumours.
Methods and results: We analysed the gene expression profiles of a total of 30 tumour samples (four WT GISTs and 26 mutated GISTs), selected the genes most differentially expressed ($P < 0.001$:448 probe sets) and validated these results by quantitative polymerase chain reaction (PCR) and immunohistochemistry. In addition, we conducted a meta-analysis merging data from human GISTs with a genomic data set from murine ICCs. The gene expression profiles of WT and mutated GISTs differed profoundly, especially in the expression of those genes restricted primarily to neural tissues. We found that mature ICCs are more similar to mutated GISTs than WT GISTs.
Conclusions: WT GISTs have different genomic profiles from both mutated GISTs and murine mature ICCs. Considering that IGF1R expression is common to both WT GISTs and putative precursor ICCs, this study suggests that WT GISTs may derive either from ICCs at a different step of differentiation or from a different cell of origin.

Keywords: gene expression profiling, GIST, ICC, ICC mature, ICC precursor, IGF1R, KIT, PDGFRA

Abbreviations: CDH2, cadherin-2; DMP, deep muscular plexus; ELAVL3, embryonic lethal, abnormal vision, Drosophila-like 3; GIST, gastrointestinal stromal tumours; ICC, interstitial cells of Cajal; IGF1R, insulin-like growth factor 1 receptor; IHC, immunohistochemistry; *KIRREL3*, Kin of IRRE-like protein 3; *Lhx2*, LIM/homeobox protein; MY, myenteric plexus; NEFL, neurofilament light polypeptide gene; NRCA, neuronal cell adhesion molecule; NSE, neurone-specific enolase; PCR, polymerase chain reaction; PDGFRA, platelet-derived growth factor receptor, alpha; TKI, tyrosine kinase inhibitor; WT, wild-type

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Introduction

Gastrointestinal stromal tumours (GISTs) are rare malignancies originating from the interstitial Cajal cells (ICCs) of the gastrointestinal tract.[1] Most GISTs are characterized by a mutation in the KIT and PDGFRA receptors, which is the pathogenic event leading to up-regulated tyrosine kinase activity.[2,3] Treatment with tyrosine kinase inhibitors (TKIs) is the standard therapeutic approach for patients with advanced or unresectable disease.[4,5] However, approximately 7–10% of GISTs do not harbour any mutations and are defined as wild-type (WT) lesions. WT GISTs are more common in children but also develop in adults, and are usually more resistant to TKIs.[6–9] The gene expression profile and gene copy number of WT GISTs have been found to be completely different from mutant GISTs.[7,10] Moreover, interesting recent data have shown that insulin-like growth factor 1 receptor (IGF1R) is up-regulated, especially in children and young adult WT patients.[7,10–12] The differential expression of IGF1R between mutated and non-mutated GISTs suggests a different differentiation step in ICC cells. Lorincz *et al.*[13,14] showed recently that murine ICC precursors are a rare IGF1R-positive, $Kit^{(low)}$, $CD44^{(+)}$, $CD34^{(+)}$, $Insr^{(+)}$ cell population retained in postnatal life that is dependent on IGF signalling for survival and differentiation. The committed ICC progenitor population has an IGF1R-positive, $Kit^{(high)}$, $CD44^{(+)}$, $CD34^{(+)}$, $Insr^{(+)}$ profile, and mature ICCs are an IGF1R-negative, $Kit^{(high)}$, $CD44^{(+)}$, $CD34^{(-)}$, $Insr^{(-)}$ cell population.[13,14] At least two classes of ICCs with functionally different gene expression profiles have been reported: the ICCs of the myenteric plexus (ICC-MY), which are the pacemaker cells, and the ICCs of the deep muscular plexus (ICC-DMP), which form synaptic connections involved in the reception and transduction of muscular neurotransmission.[16] Robinson *et al.* reported that GISTs may originate from a small subset of ICCs which is both KIT- and CD34-positive.[15] However, the exact cell type from which GISTs originate has not been identified, and the molecular and genomic background and clinical behaviour of the disease are notoriously variable. This paper aimed to compare the genome signatures of WT GISTs and mutated GISTs to disclose markers of cell differentiation and function, and thereby help to identify the cell that gives rise to these tumours.

Materials and methods

TUMOUR SAMPLES

Fresh tissue specimens of GISTs from 30 adult patients were collected from two institutions (Department of Hematology and Oncology Sciences 'L A Seragnoli', S. Orsola-Malpighi Hospital, University of Bologna, Bologna, Italy and Department of Medicine, Portland VA Medical Center and Oregon Health and Science University Knight Cancer Institute, Oregon Health and Science University, Portland, OR, USA), snap-frozen in liquid nitrogen and stored at −80°C. Patient characteristics are listed in Table 1. Mutational analysis of *KIT* (exons 9, 11, 13, 17 and 18) and *PDGFRA* (exons 12, 14 and 18) revealed that 21 patients had *KIT* point mutations (19 in exon 11 and two in exon 9, with one patient also showing a secondary mutation on exon 18), five patients had a *PDGFRA* point mutation (two in exon 12, one in exon 14 and two in exon 18) and four patients had wild-type (WT) disease. In particular, two WT GISTs (GIST-07 and GIST-10) were provided by the first institution and the diagnosis was confirmed by two experts (DS and CC) in the primary tumours, as well as in the liver and lung metastases of patient GIST-07 and in the liver and lymph nodes of patient GIST-10. Both cases were confirmed to be KIT- and DOG-1-positive GISTs. The two other WT GISTS (GIST-21 and GIST-24) were provided from the second institution; the diagnosis was confirmed by two experts (MCH and CC) and both cases were confirmed to be KIT- and DOG-1-positive.

GENE EXPRESSION

Cellular RNA was extracted using RNeasy Spin columns (Qiagen, Milan, Italy), verified for quality and labelled as outlined in the Affymetrix expression technical manual before being hybridized to U133Plus 2.0 arrays. Gene expression data were quantified by the robust multi-array analysis (*rma*) algorithm, filtered and analysed with supervised techniques using the limma modified *t*-test,[17] and were corrected for multiple testing by the Benjamini and Hochberg method for the detection of significantly differentially expressed genes between WT and mutated samples. Genes were considered to be expressed differentially at the 0.01 *P*-value cut-off level. The list of differentially expressed genes was analysed using the DAVID/Ease tool for functional classification (biological process and tissue expression). The identified markers were validated by quantitative PCR (qPCR) at the transcriptional level and by immunohistochemistry (IHC) at the protein level.

META-ANALYSIS

Gene expression data from murine ICC, both from the myenteric plexus (ICC-MY) and within the

Table 1. Patients' characteristics

ID	Age	Site	Disease status at diagnosis	Mutational status	Tumour Size	Mitotic rate
GIST_02	85	Stomach	Localized	KIT exon 11 pm V560D	8 cm	<5/50 HPF
GIST_04	79	Stomach	Localized	KIT exon 9 insertion AY502–503	5 cm	>5/50 HPF
GIST_05	68	Stomach	Localized	PDGFRA exon 12 ins/del SPDGHE566–571RIQ	>5 cm	<5/50 HPF
GIST_07	28	Stomach	Metastatic	KIT and PDGFRA WT	5–10 cm	>10/50 HPF
GIST_08	62	Stomach	Localized	KIT exon 11 pm V559D	2 cm	<5/50 HPF
GIST_09	54	Stomach	Localized	KIT exon 11 insertion TQLPYDHKWEFP574–585 at P585	2–5 cm	<5/50 HPF
GIST_10	30	Stomach	Metastatic	KIT and PDGFRA WT	>5 cm	>5/50 HPF
GIST_11	65	Stomach	Localized	KIT exon 11 deletion WK557–558	7 cm	>10/50 HPF
GIST_12	66	Stomach	Localized	PDGFRA exon pm 14 K646E	4.5 cm	<5/50 HPF
GIST_13	46	Small intestine	Localized	KIT exon 11 pm V559D	7.5 cm	<5/50 HPF
GIST_14	56	Stomach	Metastatic	KIT exon 11 homozygous deletion WK557–558	NA	NA
GIST_15	64	Stomach	Localized	PDGFRA exon 18 deletion DIMH842-845	5.5 cm	NA
GIST_16	62	Stomach	Localized	KIT exon 11 pm L576P	3.7 cm	NA
GIST_18	NA	NA	NA	KIT exon 11 pm V559G	NA	NA
GIST_20	38	Small intestine	Metastatic	KIT exon 11 deletion MYEQW552–557 Z + KIT exon 18 pm A829P	>10 cm	NA
GIST_21	25	Stomach	NA	KIT and PDGFRA WT	NA	NA
GIST_22	76	Stomach	NA	PDGFRA exon 18 pm D842V	9.5 cm	NA
GIST_23	47	Stomach	NA	KIT exon 11 pm V559D	6.4 cm	>5/50 HPF
GIST_24	18	Stomach	Metastatic	KIT and PDGFRA WT	23 cm	NA
GIST_25	84	NA	NA	KIT exon 11 deletion WKV557–559F	10 cm	>5/10 HPF
GIST_26	49	Stomach	Localized	PDGFRA exon 12 pm V561D	2 cm	NA
GIST_28	87	NA	NA	KIT exon 11 pm W557G	NA	NA
GIST_121	72	Stomach	Localized	KIT exon 11 pm V559D	5.5 cm	<5/50 HPF
GIST_124	72	Stomach	Localized	KIT exon 11 insertion 1765–1766	>10 cm	>10/50 HPF

Table 1. (*Continued*)

ID	Age	Site	Disease status at diagnosis	Mutational status	Tumour Size	Mitotic rate
GIST_125	49	Stomach	Localized	KIT exon 11 pm W557R	6 cm	5/50 HPF
GIST_129	60	Stomach	Localized	KIT exon 11 del/ins Y553–V559L	>10 cm	>10/50 HPF
GIST_130	79	Stomach	Localized	KIT exon 9 insertion A502_Y503	7.5 cm	>10/50 HPF
GIST_131	58	Small intestine	Localized	KIT exon 11 deletion V569_Y578	6 cm	<5/50 HPF
GIST_134	65	Stomach	Localized	KIT exon 11 homozygous pm V559D	>10 cm	>5/50 HPF
GIST_135	60	Stomach	Localized	KIT exon 11 deletion W557_E561	3.5 cm	>5/50 HPF

GIST, gastrointestinal stromal tumours; HPF, high-power field; NA, not applicable; WT, wild-type.

deep-muscular plexus (ICC-DMP), were downloaded from the Gene Expression Omnibus (GEO) website (Accession no. GSE7809). CEL files (Affymetrix Cell Intensity files) were normalized with *rma*, and probe sets were retained only if expression was >5 (in the $\log_2$ scale) in at least two samples. Orthologue probe sets between mouse 430 2.0 and human U133 Plus 2.0 arrays were selected based on the guidelines in Annotation Release 30 of the Affymetrix orthologue mapping repository. Human GIST and murine ICC data were merged and an unsupervised analysis was performed by the principal component dimension reduction technique (TiGR MeV).

QPCR

Total RNA was reverse-transcribed using Superscript II (Invitrogen Life Technologies, Monza, Italy) with oligo-dT primers, according to the manufacturer's guidelines. Gene-specific primers were designed with the Primer Express 3.0 Software program (Applied Biosystems, Foster City, CA, USA) and qPCR was performed using FastStart Sybr Green (Roche, Penzberg, Germany) on a LightCycler 480 apparatus (Roche). The delta delta C_t (DDC_t) method was used to quantify gene product levels relative to the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) housekeeping gene. Statistical significance was calculated using the Mann–Whitney *U*-test.

IHC

Formalin-fixed, paraffin-embedded samples for IHC were available in only two WT patients and seven mutated cases. Monoclonal anti-neurone-specific enolase (NSE) (clone E27; Roche), monoclonal anti-N-cadherin (clone 6G11; DakoCytomation, Glostrup, Denmark) and polyclonal rabbit anti-neuronal cell adhesion molecule (NRCAM) (Atlas Antibodies AB, Stockholm, Sweden) antibodies and antiserum were used. Sections were processed in a Ventana automated immunostainer (BenchMark XT, Ventana Medical Systems, Tucson, AZ, USA) using a monoclonal anti-NSE antibody. For IHC analyses using other antibodies, sections were dewaxed, rehydrated and subjected to the appropriate antigen retrievaltreatment. After cooling at room temperature, the activity of endogenous peroxidases was inhibited using methanol/H_2O_2 (0.5%) for 20 min. The sections were then washed in phosphate-buffered saline (PBS) (pH 7.2–7.4) and incubated with monoclonal/polyclonal reagent overnight in a moist chamber at RT. The sections were then washed in PBS and treated using the Novolink Polymer detection system (Novocastra, Newcastle upon Tyne, UK) according to the manufacturer's instructions.

Results

GENE EXPRESSION

The gene expression profile was profoundly different in WT compared with mutated GISTs. A total of 3250 probe sets were expressed differentially at the 0.05 *P*-value cut-off level after correcting for multiple testing by the Benjamini and Hochberg method. To gain insight into this difference, we selected the top genes that were expressed highly differentially ($P < 0.001$: 448 probe sets) and ran the David/EASE tool with

Table 2. Gene ontology – biological process; the functional categories related to neurogenesis are shown in bold type

Term	% genes	*P*-value	Fold enrichment
GO:0007416 synaptogenesis	**1.58**	**0.0017**	**9.62**
GO:0050808 synapse organization	**1.89**	**0.0035**	**5.86**
GO:0055114 oxidation reduction	5.99	0.0127	1.87
GO:0030036 actin cytoskeleton organization	3.15	0.0129	2.66
GO:0030030 cell projection organization	**4.10**	**0.0154**	**2.20**
GO:0048666 neurone development	**3.79**	**0.0185**	**2.24**
GO:0006914 autophagy	1.26	0.0216	6.65
GO:0016337 cell–cell adhesion	3.15	0.0294	2.30
GO:0048747 muscle fibre development	**1.58**	**0.0331**	**4.10**
GO:0042551 neurone maturation	**0.95**	**0.0336**	**10.26**
GO:0007243 protein kinase cascade	3.79	0.0398	1.98
GO:0007528 neuromuscular junction development	**0.95**	**0.0486**	**8.39**
GO:0031175 neurone projection development	**2.84**	**0.0525**	**2.20**

Table 3. Tissue expression; tissues of neural origin are shown in bold type

Term	Number of genes	% genes	*P*-value	Fold enrichment
Hippocampus	**31**	**9.78**	**6.05E-05**	**2.23**
Brain	**166**	**52.37**	**3.94E-04**	**1.22**
Alzheimer cortex	**8**	**2.52**	**0.002107**	**4.47**
Amygdala	**28**	**8.83**	**0.010265**	**1.66**
Pancreas	26	8.20	0.012295	1.68
Liver	48	15.14	0.015983	1.39
Foetal brain	**21**	**6.62**	**0.025363**	**1.68**

respect to gene ontology (Table 2) and tissue expression (Table 3). Both analyses showed that neurogenesis-related functions remained significant, and that the pattern of expression of the genes up-regulated in WT GISTs was restricted mainly to neural tissues.

META-ANALYSIS

To gain insight into the differential neural commitment of GIST tumours, we conducted a meta-analysis on data from murine ICCs as published in Physiol. Gen. 2007, merged data from human GISTs and murine ICCs (DMP and MY), and conducted a principal component analysis (Figure 1). Surprisingly, murine ICC samples did not cluster independently from human GISTs, but clustered with human tumours harbouring either *KIT* or *PDGFRA* mutations, which confirmed the origin of these tumours from this cell lineage. In addition, WT GISTs clustered together and lay further from mutated tumours and ICCs. This suggested that ICCs have a gene expression signature much more similar to mutated GISTs than to WT GISTs and suggested, conversely, that the expression profile of WT GISTs is not shared either by mutated tumours or by the putative precursor ICCs.

VALIDATION BY QPCR AND IHC

Several neural markers were validated by qPCR and by IHC. Using qPCR we confirmed the differential expression of the top scoring genes encoding structural proteins expressed in neural tissues, such as cadherin-2 (CDH2) and neurofilament light polypeptide gene (NEFL), and of neural progenitor-specific genes [IGF1R, LIM/homeobox protein (*LHX2*), kin of IRRE-like protein 3 (*KIRREL3*) and embryonic lethal, abnormal vision, Drosophila-like 3 (ELAVL3)]. We found that all these neural markers were up-regulated markedly, and some were expressed selectively in WT GISTs (Figure 2).

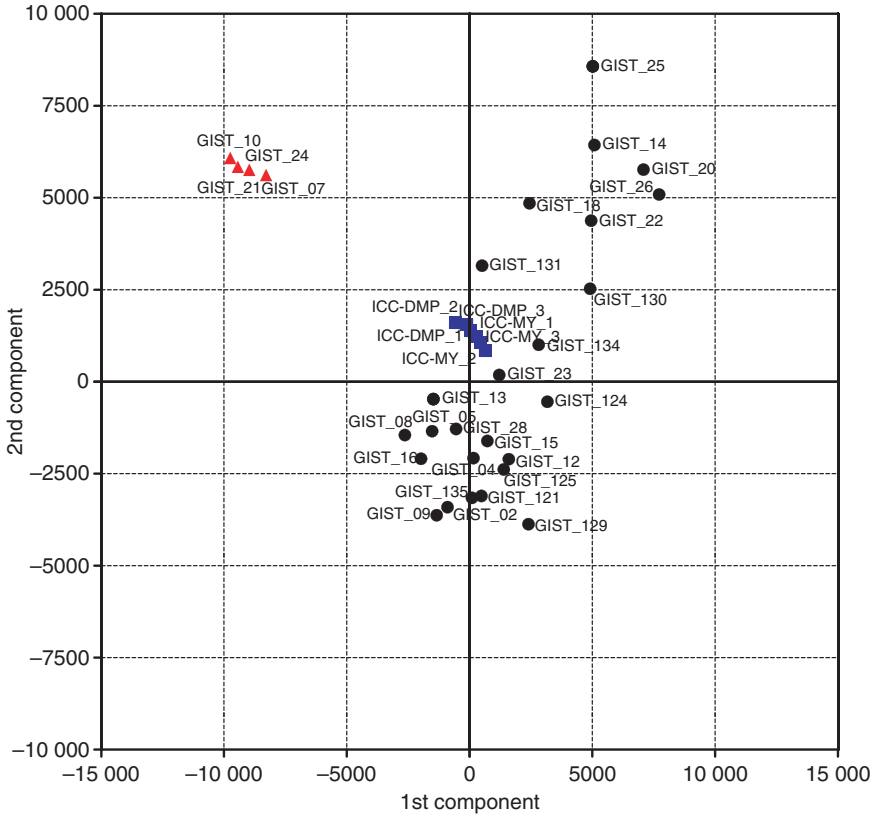

Figure 1. Principal component analysis of the merged dataset of human gastrointestinal stromal tumours (GIST) samples and murine (ICCs). Mutated GISTs (black) co-map with ICCs (blue), while WT GISTs (red) have a different profile.

IHC analysis showed that both WT GIST tumours overexpressed NSE, CDH2 and NEFL, and that none of the seven mutated GISTs overexpressed these genes (Figure 3).

Discussion

The gene expression profile of WT GISTs was profoundly different from mutated GISTs. The expression of the top differentially expressed genes ($P < 0.001$: 448 probe sets) was restricted mainly to neural tissues and suggested that neurogenesis-related functions are dominant. Several neural markers were validated by qPCR and IHC. Moreover, the present meta-analysis conducted on data in murine ICCs published in Physiol. Gen. 2007[16] and on data from human GISTs and murine ICCs (DMP and MY) showed that mutated GISTs co-map primarily with ICCs, and confirmed that WT GISTs have a distinctive genomic profile. The comparison of genomic profiles between WT and mutated GISTs and the ICC classes may shed light on the cellular origins and oncogenic events contributing to the formation of these tumours.

To characterize the physiology and origins of newly mature ICCs, Lorincz *et al.*[13] evaluated freshly dissected and cultured muscles of murine stomach. They identified a rare cell population characterized by an IGF1R-positive, Kit$^{(low)}$, CD44$^{(+)}$, CD34$^{(+)}$, Insr$^{(+)}$ phenotype that may be considered the precursor ICC. The survival of these cells was dependent on stem cell factor (SCF), and their differentiation into mature ICCs (both into the main mature ICC classes) was dependent on IGF1. Therefore, IGF1 is a factor that permits the maturation of precursor ICCs, after which ICCs lose IGF1R expression but maintain CD34 expression, thereby displaying the same characteristics of mature ICCs in postnatal tissues, which do not express insulin receptor (*INSR*)/IGF1R and express CD34.[13] As discussed by Huiziniga and white[14] tumour development may be initiated when the cellular self-renewal process loses control of molecular deregulation, such as impairment of the IFG1R pathway. Several findings from the present study suggest two potential hypotheses on the cell of origin of WT GISTs. The first of these findings is that a subset of GISTs may develop when the IGF1R-dependent balance between cell renewal and differentiation is disrupted. Secondly, WT GISTs differ from mutated GISTs due to the absence of the *KIT/PDGFRA* mutation and to differential IGF1R expression. WT GISTs present the expression of IGF1R as well as the 'precursor' ICCs, and 'mature' ICCs have a gene expression profile that resembles more closely mutated GISTs than WT GISTs.

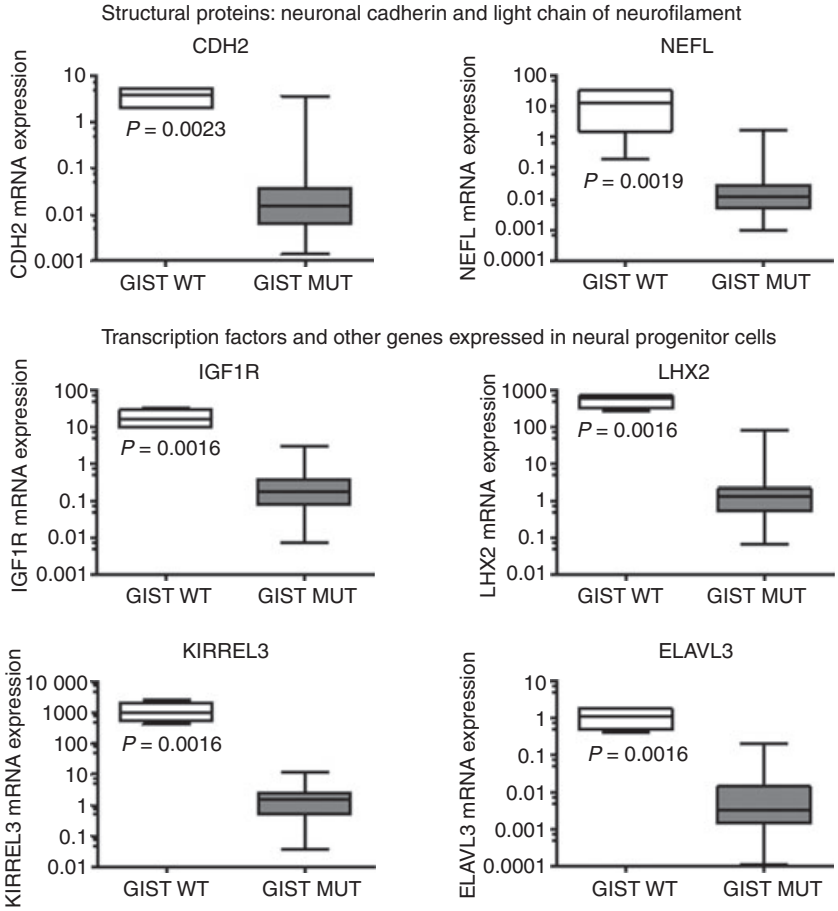

Figure 2. Expression of neural markers normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression as evaluated by quantitative polymerase chain reaction (qPCR). Statistical significance was calculated by the Mann–Whitney *U*-test.

Finally, WT and mutant GISTs have different molecular profiles of several genes associated with neural commitment.

The first hypothesis suggested by these findings is that because these tumour types share IGF1R expression, WT GISTs may derive from ICCs in a different differentiation step which are more similar to that of the 'precursor' ICCs as described by Lorincz *et al.* In contrast, *KIT/PDGFRA* mutations are oncogenic events occurring in mature ICCs and are considered to be responsible for the development of mutated GISTs.

The second hypothesis based on the present findings is that WT GISTs may derive from a cell of mesenchymal origin with neural differentiation. Indeed, neural differentiation in sarcomas, which are also derived from the mesenchymal lineage, is not uncommon: Ewing sarcomas are dependent on IGF1R signalling for proliferation and survival,[18] and display clear neural commitment driven by the oncogenic EWS-FLI1 fusion protein.[19,20] In contrast, clear cell sarcomas of the kidney show marked up-regulation of neural markers, many of which are common to WT GISTs.[21] Therefore, it is likely that WT GISTs and mutated GISTs are two tumours originating from two distinct cell types, or are generated by two different mechanisms.

The multistep pathogenesis of the mesenchymal-derived GISTs remains poorly understood, in contrast to epithelial-derived tumours such as gastric and colonic cancers, in which the morphological and molecular steps of carcinogenesis have been characterized more clearly. However, limited data are available on the occurrence of putative precursor lesions with different clinical pathological characteristics, and on incidental and/or multifocal small GISTs.[22–28] Unexpectedly, one study found that sporadic Cajal cell hyperplasic regions (CD34-positive/KIT-positive) were common in the distal region of apparently normal oesophageal mucosa in oesophageal cancer patients.[22] Corless *et al.*[23] reported GISTs that were discovered at autopsy or during endoscopy or laparotomy, and which ranged in size from 4 to 10 mm. Most of these lesions presented KIT mutations and, in particular, 77% of these tumours had KIT exon 11 mutations and approximately 8% had *KIT* exon 9 mutations, together suggesting that acquisition of an activating mutation

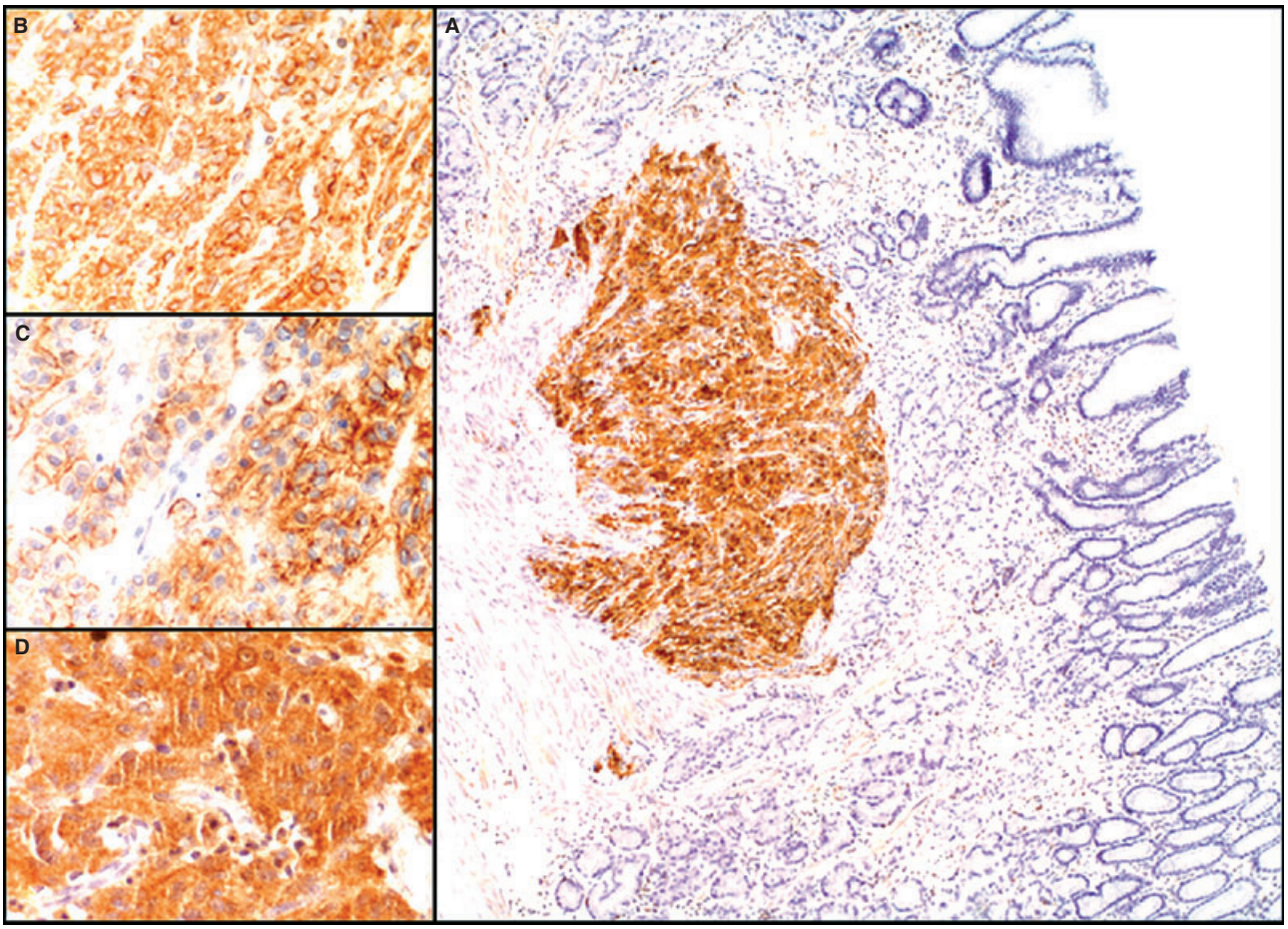

Figure 3. Interstitial cells of Cajal (IHC) images of a wild-type (WT) gastrointestinal stromal tumours (GIST). **A**, Neurone-specific enolase (NSE) immunostaining; GIST cells coexpressed N-cadherin (**B**), anti-neuronal cell adhesion molecule (NRCAM) (**C**) and NSE (**D**).

occurs very early in tumour development. Agaimy *et al.*[24] described lesions called 'GIST tumourlets' found in the proximal stomach during the autopsy of patients aged 50 years or older, and the authors suggested that these tumourlets had a very limited potential to progress to clinical GISTs. In addition, Agaimy *et al.*[25] described multifocal sporadic GISTs of the stomach located within 4 cm of each other; 79% of the tumours had *KIT* exon 11 mutations that varied across tumours within the same patient, and that the remainder of these tumours were WT. The authors postulated that multifocal GISTs may originate from a distinct subset of stem cells or a subset of mutated ICCs that grow independently. Gasparotto *et al.*[26] also reported that a fraction of GIST patients present a multiplicity of lesions harbouring KIT or PDGFRA mutations, with only one WT case observed, suggesting that widespread tumour precursor cells give rise to these tumours. Finally, distinct KIT mutations were also documented in microdissected ICCs of normal appearance in the muscularis propria that were localized close to GISTs.[29] Most of these studies concluded that the high frequency of KIT mutations observed both in sporadic or multifocal small GIST or ICC hyperplasias were because these mutations occur very early in tumorigenesis, and are required for GIST development. Unfortunately, few of these studies conducted a complete analysis of all *KIT* or *PDGFRA* exons, so that the incidence of true WT precursor lesions remains unclear and no data on IFG1R or other molecular phenotypes are available. Because the incidence of precursor lesions is high but GISTs are rare, complete tumour regression may be common;[30] therefore, molecular mechanisms or genetic/epigenetic alterations other than *KIT/PDGFRA* kinase mutations must also be considered when evaluating the mechanisms of GIST tumorigenesis. Although these studies add to our knowledge of how mutated GISTs develop, the mechanisms involved in the pathogenesis and progression of WT GISTs remain unclear.

Future studies will identify the origin and molecular deregulation of WT GISTs. Because WT GISTs express many neural-commitment transcription factors and other markers such as LHX2 (as shown in Figure 2), one interesting analysis would be to correlate the genome profiles of WT GIST and precursor ICCs to determine whether this rare population of cells also displays this genetic profile. In addition, the genomic profiles of other mesenchymal tumours with established neural commitment lineages should be compared to WT GISTs. These studies will be crucial to identify therapeutic targets and to develop translational therapies for neural-committed tumours to WT GIST, which are usually less responsive to TKIs inhibitors. In addition, inhibition of the IGF1R pathway in WT GISTs should be encouraged.

In conclusion, we found that WT GISTs have different genomic profiles from mutated GISTs, especially in the expression of neural-commitment transcription markers, and that mature ICCs are similar to mutated GISTs but not WT GISTs. This study suggests that WT GISTs may be derived from different non-ICC cell types, or may derive from ICCs during a different differentiation step, such as from precursor ICCs. Further studies are needed to further the knowledge of WT GIST pathogenesis and to identify appropriate treatment for patients with these tumours.

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References

1. Streutker CJ, Huizinga JD, Driman DK, Riddell RH. Interstitial cells of Cajal in health and diseases. Part II: ICC and gastrointestinal stromal tumors. *Histopathology* 2007; **50;** 190–202.
2. Hirota S, Isozaki K, Moriyama Y *et al.* Gain of function mutations of c-kit in human gastrointestinal stromal tumors. *Science* 1998; **279;** 577–580.
3. Lux ML, Rubin BP, Biase TL *et al.* KIT extracellular and kinase domain mutations in gastrointestinal stromal tumors. *Am. J. Pathol.* 2000; **156;** 791–795.
4. Demetri GD, von Mehren M, Blanke CD *et al.* Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumours. *N. Engl. J. Med.* 2002; **347;** 472–480.
5. Demetri GD, van Oosteroom AT, Garrett CR *et al.* Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: a randomised controlled trial. *Lancet* 2006; **368;** 1329–1338.
6. Janewy KA, Pappo AS. Pediatric gastrointestinal stromal tumor. *Hematol. Oncol. Clin. North Am.* 2009; **23;** 15–34.
7. Agaram NP, Laquaglia MP, Ustun B *et al.* Molecular characterization of pediatric gastrointestinal stromal tumors. *Clin. Cancer Res.* 2008; **14;** 3204–3215.
8. Heinrich MC, Corless CL, Demetri GD *et al.* Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumor. *J. Clin. Oncol.* 2003; **21;** 4342–4349.
9. Heinrich MC, Corless CL, Blanke CD *et al.* Molecular correlates of imatinib resistance in gastrointestinal stromal tumors. *J. Clin. Oncol.* 2006; **24;** 4764–4774.
10. Pantaleo MA, Astolfi A, Di Battista M *et al.* Insulin-like growth factor 1 receptor (IGF1r) expression in wild-type GIST: a potential novel therapeutic target. *Int. J. Cancer* 2009; **125;** 2991–2994.
11. Tarn C, Rink L, Merkel E *et al.* Insulin-like growth factor 1 receptor is a potential therapeutic target for gastrointestinal stromal tumors. *Proc. Natl Acad. Sci. USA* 2008; **105;** 8387–8392.
12. Janeway KA, Zhu MJ, Barretina J, Perez-Atayde A, Demetri GD, Fletcher JA. Strong expression of IGF1R in pediatric gastrointestinal stromal tumors without IGF1R genomic amplification. *Int. J. Cancer* 2010; **27;** 2718–2722.
13. Lorincz A, Redelman D, Horváth VJ, Bardsley MR, Chen H, Ordög T. Progenitors of interstitial cells of Cajal in the postnatal murine stomach. *Gastroenterology* 2008; **134;** 1083–1093.
14. Huizinga JD, White EJ. Progenitors cells of interstitial cells of Cajal: on the road to tissue repair. *Gastroenterology* 2008; **134;** 1252–1253.
15. Robinson TL, Sircar K, Hewlett BR, Chorneyko K, Riddell RH, Huizinga JD. Gastrointestinal stromal tumors may originate from a subset of CD34-positive interstitial cells of Cajal. *Am. J. Pathol.* 2000; **156;** 1157–1163.
16. Chen H, Ordög T, Chen J *et al.* Differential gene expression in functional classes of interstitial cells of Cajal in murine small intestine. *Physiol. Genomics* 2007; **31;** 492–509.
17. Smyth GK. Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. *Stat. Appl. Genet. Mol. Biol.* 2004; **3;** Article 3, DOI: 10.2202/1544-6115.1027
18. Scotlandi K, Picci P. Targeting insulin-like growth factor 1 receptor in sarcomas. *Curr. Opin. Oncol.* 2008; **20;** 419–427.
19. Hu-Lieskovan S, Zhang J, Wu L, Shimada H, Schofield DE, Triche TJ. EWS-FLI1 fusion protein up-regulates critical genes in neural crest development and is responsible for the observed phenotype of Ewing's family of tumors. *Cancer Res.* 2005; **65;** 4633–4644.
20. Rocchi A, Manara MC, Sciandra M *et al.* CD99 inhibits neural differentiation of human Ewing sarcoma cells and thereby contributes to oncogenesis. *J. Clin. Invest.* 2010; **120;** 668–680.
21. Cutcliffe C, Kersey D, Huang CC, Zeng Y, Walterhouse D, Perlman EJ. Clear cell sarcoma of the kidney: up-regulation of neural markers with activation of the sonic hedgehog and Akt pathways. *Clin. Cancer Res.* 2005; **11;** 7986–7994.
22. Agaimy A, Wünsch PH. Sporadic Cajal cell hyperplasia is common in resection specimens for distal oesophageal carcinoma. A retrospective review of 77 consecutive surgical resection specimens. *Virchows Arch.* 2006; **448;** 288–294.
23. Corless CL, McGreevey L, Haley A, Town A, Heinrich MC. KIT mutations are common in incidental gastrointestinal stromal tumors one centimeter or less in size. *Am. J. Pathol.* 2002; **160;** 1567–1572.
24. Agaimy A, Wünsch PH, Hofstaedter F *et al.* Minute gastric sclerosing stromal tumors (GIST tumorlets) are common in adults and frequently show c-KIT mutations. *Am. J. Surg. Pathol.* 2007; **31;** 113–120.
25. Agaimy A, Dirnhofer S, Wünsch PH, Terracciano LM, Tornillo L, Bihl MP. Multiple sporadic gastrointestinal stromal tumors

(GISTs) of the proximal stomach are caused by different somatic KIT mutations suggesting a field effect. *Am. J. Surg. Pathol.* 2008; **32;** 1553–1559.
26. Gasparotto D, Rossi S, Bearzi I *et al.* Multiple primary sporadic gastrointestinal stromal tumors in the adult: an underestimated entity. *Clin. Cancer Res.* 2008; **14;** 5715–5721.
27. Haller F, Schulten HJ, Armbrust T, Langer C, Gunawan B, Füzesi L. Multicentric sporadic gastrointestinal stromal tumors (GISTs) of the stomach with distinct clonal origin: differential diagnosis to familial and syndromal GIST variants and peritoneal metastasis. *Am. J. Surg. Pathol.* 2007; **31;** 933–937.
28. Kang DY, Park CK, Choi JS *et al.* Multiple gastrointestinal stromal tumors: clinicopathologic and genetic analysis of 12 cases. *Am. J. Surg. Pathol.* 2007; **31;** 224–232.
29. Ogasawara N, Tsukamoto T, Inada K *et al.* Frequent c-Kit gene mutations not only in gastrointestinal stromal tumors but also in interstitial cells of Cajal in surrounding normal mucosa. *Cancer Lett.* 2005; **230;** 199–210.
30. Rossi S, Gasparotto D, Toffolati L *et al.* Molecular and clinicopathologic characterization of gastrointestinal stromal tumors (GISTs) of small size. *Am. J. Surg. Pathol.* 2010; **34;** 1480–1491.