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CD133 and CD44 are universally overexpressed in GIST and do not represent cancer stem cell markers

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Abstract

Although imatinib mesylate has been a major breakthrough in the treatment of advanced GIST, complete responses are rare and most patients eventually develop resistance to the drug. Thus the possibility of an imatinib-insensitive cell subpopulation within GIST tumors, harboring stem cell characteristics, may be responsible for the clinical failures. However, the existence of a cancer stem cell component in GIST has not been yet established. The present study was aimed to determine whether expression of commonly used stem cell markers in other malignancies, i.e. CD133 and CD44, might identify cells with characteristics of cancer stem/progenitor cells in human GIST. CD133 and CD44 expression in GIST explants was analyzed by flow cytometry, immunofluorescence, and gene expression. Their transcription levels were correlated with clinical and molecular factors in a large, well-annotated cohort of GIST patients. FACS sorted GIST cells based on CD133 and CD44 expression were isolated and used to assess phenotypic characteristics, ability to maintain their surface expression, sensitivity to imatinib, and expression signature. The enrichment in CD133/CD44 cells in the side population (SP) assay was also investigated. CD133 expression was consistently found in GIST. CD133⁺ cells formed more colonies, were more invasive in a matrigel assay, and showed enrichment in the SP cells, compared to CD133⁻ cells. CD133 expression was also detected in the two imatinib-sensitive GIST cell lines, while was absent in the imatinib-resistant lines. Our results show that CD133 and CD44 are universally expressed in GIST, and may represent a lineage rather than a cancer stem cell marker.

Keywords

gastrointestinal stromal tumor; CD133; CD44; cancer stem cells; gene expression; KIT

INTRODUCTION

Most gastrointestinal stromal tumors (GIST) express KIT and show activating mutations in either *KIT* or less commonly in *PDGFRA*. Although a partial response or stable disease is achieved in ~80% of advanced GIST patients treated with imatinib mesylate, complete responses are rare and most patients develop resistance to the drug. Even in patients responding to imatinib therapy who undergo elective surgery, the microscopic examination of their tumors reveals viable tumor cells, with variable degrees of proliferative activity (Agaram et al., 2007). It is possible that these residual foci of viable tumor escaping imatinib-triggered apoptosis signals are enriched in cells with stem cell properties. It is also likely that secondary genetic events occurring in these viable tumor foci establish the foundation for imatinib-resistance. As such, the most common mechanism of resistance is the occurrence of a 2nd site *KIT* mutation in half of the patients (Antonescu et al., 2005; Debiec-Rychter et al., 2005). In the remaining cases however, the mechanisms of drug failure are not well defined and questions still remain on the role of an imatinib-insensitive stem cell component in triggering drug-resistance.

Cancer stem cells (CSCs) are defined as a population of undifferentiated tumor cells responsible for tumor initiation, maintenance and spreading (Pardal et al., 2003). These 'tumor initiating cells' exhibit the abilities of self-renewal, differentiation, metastasis, and insensitivity to traditional antitumor therapies. Although CSCs have been isolated and characterized in certain types of leukemia, brain tumors, breast and colon cancers, their existence and role in driving sarcomagenesis has not been well established. Furthermore, the existence of a cancer stem cell component in GIST has not been demonstrated yet.

For solid tumors, the repertoire of cell surface markers currently used to identify human CSCs includes among others CD133 and CD44. CD133 (also known as Prominin-1 or AC133) expression has been widely used in a number of cancers (Tirino et al., 2009; Zhu et al., 2010; Yan et al., 2011). CD133 is a transmembrane pentaspan protein, which was initially described as a surface antigen specific for human hematopoietic stem cells (Miraglia et al., 1997; Yin et al., 1997) and as a marker for murine neuroepithelial cells and several other embryonic epithelia (Weigmann et al., 1997). Although the biological function of CD133 remains unknown, CD133 alone or in combination with other markers is currently used for the isolation of stem cells from numerous normal tissues, as well as the identification and isolation of a putative cancer stem cell population from a variety of malignant tumors, including gliomas and epithelial malignancies (Singh et al., 2003; Collins et al., 2005; Ricci-Vitiani et al., 2007).

Although two recent reports showed high expression levels of CD133 in GIST, no functional experiments were carried out to elucidate the role of CD133 and other potential CSC markers in GIST (Arne et al., 2011). Furthermore, Arne et al suggested an association between CD133 and a poor outcome by univariate analysis. Thus, the present study was designed and conducted with the aim to determine whether CD133 and CD44 are expressed in human GIST, and whether CD133^{+/−} and/or CD44^{+/−} sorted cells from primary tumors and imatinib sensitive/resistant GIST cell lines manifest any of the currently recognized stem/progenitor cell properties, and whether their expression may correlate with clinically and molecular relevant subsets of GIST. In addition we used the side population (SP) assay to investigate CSCs in GIST explants and an imatinib-sensitive GIST cell line, as this method has been recently used with success to isolate CSC in leukemia and other malignancies. Putative GIST CSC subpopulations, isolated by these various techniques were then compared for differences in proliferation, colony assays, and gene expression profiling.

MATERIAL AND METHOD

Explanted GIST tumor samples

Fresh tumor tissue collected from surgical specimens of primary, imatinib-naïve GIST tumors (IRB protocol # 02-060) was minced with a scalpel at room temperature and enzymatically disaggregated with collagenase 1A (Sigma) at 37°C for one hour into single-cell suspension. Cells were maintained in RPMI1640 containing 15% FCS, 0.5% Mito, and 3% Bovine Pituitary Extract (BD Biosciences, Bedford, MA) for 24–48 hours. Cells were detached with TrypLE Express (Invitrogen) and resuspended in sorting buffer (PBS containing 2% FCS, 0.1 mM EDTA and 1% penicillin/ streptomycin) and sorting stained with anti-CD133 (Miltenyi Biotec), anti-CD44 (eBiosciences), and anti-KIT (BD Biosciences). No other functional assays were possible using the GIST explanted tissue.

GIST Cell Lines

Four human primary GIST cell lines were used for *in-vitro* experiments: GIST882, GIST48, GIST62 (kindly provided by Dr. Jonathan Fletcher, Dana-Farber Cancer Institute, Boston, MA) and GIST T1 (kindly provided by Dr. Takahiro Taguchi, from Kochi University, Nankoku, Kochi, Japan), see Table 1. GIST882, GIST62 and GIST-T1 were grown in RPMI containing 15% FCS, while GIST48 were grown in F10 containing 15% FCS, 0.5 % Mito+ serum extender and 1% Bovine pituitary extract (BD Biosciences).

Immunofluorescence

Immunofluorescence staining was performed on chamber slides for the cell lines and on two tissue microarrays (TMA) of formalin-fixed, paraffin-embedded tissue, encompassing 120 GIST tumors from 114 patients of various anatomic locations, genotypes, including imatinib-naïve and imatinib-resistant tumors. Each tumor was represented by two cores and in six patients tissue cores from different surgical procedures were included. The clinical and molecular characteristic of the 114 patients included on the TMA are the following: anatomic location: 52 stomach, 45 small bowel, 7 rectum; mutation: 97 KIT exon 11, 8 KIT exon 9, 5 PDGFRA exon18 and 4 wild type; primary vs metastatic: 96 primary tumors and 24 metastatic lesions. Twelve patients were treated with imatinib and their samples included in the TMA were imatinib-sensitive in 10 cases and imatinib-resistant in 2 cases. The slides were stained with anti-CD133 (Miltenyi Biotec) and CD44 (eBiosciences), followed by Alexa-488 or Alexa-594 conjugated secondary antibodies (Molecular Probes, Eugene, OR). Slides were examined with a Zeiss Axioplan 2 or Leica TCS SP2 AOBS microscopes. In addition the hyperplastic interstitial cells of Cajal (ICC) within the myenteric plexus from two familial GIST patients were investigated for CD133 expression.

Flow Cytometry and Cell Sorting

Cultured cells were harvested with TrypLE Express (Invitrogen) and resuspended in sorting buffer (PBS containing 2% FCS, 0.1 mM EDTA and 1% penicillin/streptomycin) for analysis or sorting. Cells were stained with combinations of fluorochrome-conjugated monoclonal antibodies against human AC133 (Miltenyi Biotec), CD44 (eBiosciences) and KIT (BD Biosciences), at concentrations recommended by the manufacturers and incubated at 4°C in the dark for 30 minutes. Dead cells were eliminated with DAPI. The labeled cells were analyzed and separated by a Moflo cell sorter (Beckman Coulter) and data was analyzed with FlowJo software version 7.2 (Tree Star, Ashland, OR).

Single-cell suspensions obtained from tumor explants were prepared with a similar staining procedure as above. GIST tumor cells were identified with KIT expression. Mast cells, typically expressing both KIT and CD45, were gated out with an anti-CD45 antibody (BD Biosciences).

For the cell cycle and side population (SP) cell assays the cells were suspended in 15µg/ml and 5µg/ml, respectively, culture media with Hoechst 33342 (Invitrogen). Cells were incubated at 37°C for 90 min. Staining with CD133 and CD44 antibodies was performed as described above. Cells were labeled with 1 µg/ml propidium iodide to assess viability in SP cells by FACS. Cells from the SP and non-SP fractions were collected for microarray analysis.

Cell Proliferation Assays

Cell proliferation was monitored dynamically by using the RT-CES system (ACEA Biosciences, CA). Cells were seeded at 10,000 cells/well in ACEA's 16 × E-plate. Cell proliferation was monitored every 15 minutes for at least three days and assayed at different endpoints (1, 2 or 3 days) by using the standard Cell Titer MTS assay (Promega).

Colony Formation Assay

Cells were plated at a density of 100–500 cells/well, in 96-well plates and cultured for 3–14 days. Cells were fixed in 5% Glutaraldehyde, stained with 0.5% Toluidine Blue, and counted microscopically at 10× magnification. Each experiment was replicated at three different times and for each experiment triplicates were included.

In Vitro Invasion Assay

In vitro invasion assays were performed by using the Multiwell Insert System (Becton Dickinson). Briefly, cells were serum starved for 3 hours and 50,000 cells were placed on the top insert coated with matrigel. Serum-free media or media with 15% serum was placed in the bottom well. Cells that invaded through the matrigel were identified by fixation in 5% glutaraldehyde and staining with 0.5% Toluidine Blue, and counted microscopically. Each experiment was replicated at three different times and for each experiment triplicates were included.

Microarray Analysis

Affymetrix U133A chip platform microarray data on 131 GISTs and 25 soft tissue sarcomas, encompassing 6 different histologic subtypes (5 leiomyosarcomas, 6 fibrosarcomas, 4 clear cell sarcomas, 4 myxoid/round cell liposarcoma, 4 synovial sarcomas, 2 dedifferentiated liposarcomas) were available for mining and data analysis, as previously described (Segal et al., 2003; Antonescu et al., 2005). As a smaller amount of RNA was available from the selected subpopulations from GIST cell lines, the hybridization was performed instead on the Affymetrix U133A 2.0 chip. Data analysis was performed only among similar platform chips. Differentially expressed genes were identified by applying ANOVA with a FDR of < 0.05.

RESULTS

High expression levels of CD133 and CD44 detected in GIST compared with other soft tissue sarcomas

Transcriptional microarray data from 131 GISTs and 25 other soft tissue sarcomas, spanning six different histologic subtypes, showed a significantly higher expression of CD133 and CD44 in GIST (Fig. 1). The mRNA levels of CD133 and CD44 expression were compared among various clinical and molecular parameters (Table 1). There was no correlation between CD133 and/or CD44 mRNA expression and KIT levels, mainly because KIT mRNA was uniformly upregulated at the transcriptional level, regardless of anatomic site, presence of type of KIT mutation. CD133 expression was significantly higher in tumors located in the stomach compared to the small bowel. GIST harboring a *PDGFRA* mutation

showed a lower expression than tumors with a *KIT* mutation or lacking mutations in *KIT*/*PDGFRA*. Similarly, CD44 expression was higher in gastric GIST, pediatric and young adult wild-type GIST, but was lower in *PDGFRA* mutant-tumors. There were no significant correlations between CD133 or CD44 expression and mitotic rate or risk of malignancy in imatinib-naïve, primary GIST. A significant decrease of CD133 expression was noted in imatinib-responsive GIST, while the transcription levels were similar in imatinib-resistant and imatinib-naïve tumors.

The CD133 protein expression was also detected by immunofluorescence in the GIST TMA sections, with a diffuse and strong pattern in 95% of cores examined (Fig. 2A–C). There was no difference in intensity or degree of expression noted by immunofluorescence among different clinical/molecular GIST subsets or between imatinib-naïve versus imatinib-treated samples. In addition, CD133 expression co-localized with KIT by immunofluorescence dual staining in the hyperplastic ICC cells of a familial GIST (Fig 2 D–F). Despite many attempts, the immunofluorescence using the anti-CD44 (eBiosciences) on both GIST TMA and the hyperplastic ICCs did not yield a sharp signal to perform accurate interpretation for positive/negative results. The high level of CD133 and CD44 expression, however, was also confirmed by the high levels of CD133 and CD44 surface expression by flow cytometric analysis, gated based on KIT-positive CD45-negative population, from all 10 fresh tumor GIST explants tested. (Fig. 2G,H).

Flow cytometry was used to determine the expression of CD133 and CD44 in four currently available GIST cell lines. Considerable CD133 protein expression was detected in the two imatinib-sensitive cell lines, GIST882 and GIST-T1, while the imatinib-resistant cell lines, GIST48 and GIST62, were mainly composed of CD133⁻ cells (Table S1 and Fig. 3A–D). The ratio of CD133⁺ and CD133⁻ cells in each of these cell lines remained constant in different confluence conditions. Notably, the CD133⁺ cells were also consistently CD44⁺. By immunofluorescence, strong expression of KIT, CD133 and CD44 was detected in the GIST882 cell line (Fig. 3E–G).

Since GIST882 was the only cell line with a substantial proportion of both positive and negative subpopulations expressing CD133 and CD44, further cell sorting experiments based on these two surface makers were carried out only with this cell line. Of interest, FACS analysis of sorted cell sub-populations showed that CD133 expression remained stable after seven passages, whereas sorted CD44⁺ and CD44⁻ cells shifted to about 60–70% CD44⁺ during subsequent cultures (Fig. S1).

Morphologic and Functional Properties of CD133 and CD44 GIST cellular subsets in vitro

Flow cytometry combining cell cycle analysis and surface phenotype was performed for GIST882 and GIST-T1. An increase of G2/M and S phase was noted in the CD44⁺ cells regardless of the CD133 expression (Fig. S2A–C). In contrast, CD133⁺ cells were smaller in size and more complex in intracellular structure than CD133⁻ cells (Fig. S2D,E). Microscopically, CD133⁺ sorted cells grown on chamber slides also showed a smaller size compared with the CD133⁻ cells (Fig. S2F,G).

Both dynamic monitoring of cell proliferation using the RT-CES system and MTS proliferation assay detected the highest growth rate in the CD133⁻/CD44⁺ sorted cell subpopulation from GIST882. The doubling time was 21.4±0.35 hours in the CD133⁺/CD44⁺ cell population, while the CD133⁻/CD44⁺ cell population showed a doubling time of 13.6±0.31 hours ($p<0.0001$)(Fig. 4A,B). However, the response to imatinib treatment evaluated by dynamic monitoring of cell proliferation between the CD133⁺ and CD133⁻ subsets showed only minimal changes in their IC₅₀ (CD133⁻, 0.22–0.27μM; CD133⁺, 0.08–0.13μM)(Fig. 4C).

Among the different GIST cell lines, T1 had the greatest ability of colony formation (Fig. 4D). Colony assays showed that in GIST882, the CD133⁻/CD44⁺ cells demonstrated the greatest self-renewal ability. CD133-sorted GIST882 cells formed colonies in 7–14 days, with a plating efficiency of 5.3 ± 1.1 colonies/well in the CD133⁺ subset; and 12.5 ± 1.4 in the CD133⁻ subpopulation ($p=0.0073$) (Fig. 4E). Furthermore, the CD133⁻ cells were more invasive than CD133⁺ cells in an *in vitro* matrigel invasion assay (Fig. 4F). A significant limitation of this study is the rather small number of GIST cell lines and that functional assays could not be performed on primary culture of the explants.

GIST CD133⁻ cellular subset has the highest proportion of side population (SP) cells

In GIST882, flow cytometry showed that SP cells are mainly detected in the CD133⁻ cell population (Fig. 5). SP was reanalyzed from three to five passages after sorting. CD133⁻ cultured cells have the largest proportion of SP compared to CD133⁺ cells, even after five passages of culture.

DISCUSSION

The pathologic and molecular heterogeneity seen with imatinib response in GIST raises the possibility of a subpopulation with stem cell activity pre-existent within the overall tumor mass that remains insensitive to the drug. This small subset of tumor cells with self-renewal abilities may escape the apoptosis signals and provide the basis of imatinib resistance in GIST. Identification of a tumor progenitor population with intrinsic mechanisms for drug resistance might provide important clues for improved therapeutic intervention.

CD133 is considered an attractive therapeutic target in other cancers, since it was shown to enrich in cells with tumor-initiating ability (so-called 'cancer stem cells') in immune-deficient mice from a variety of human solid tumors (Tirino et al., 2009; Shi et al., 2010). However, the present findings, in agreement with the two recent studies (Arne et al., 2011; Bozzi et al., 2011), suggest that CD133 is universally expressed in GIST, compared to other sarcoma types. Among GIST clinical and molecular subsets, both CD133 and CD44 showed high transcriptional up-regulation in primary gastric tumors and in *KIT* mutants. *PDGFRA*-mutant GISTs showed lower levels for both CD133 and CD44. These results suggest that CD133 may be used as a potential diagnostic marker for the ICC/GIST lineage. This is not that surprising, since GISTs consistently express two other stem cell markers, KIT and CD34, which are stem cell markers in the hematopoietic lineage. Consistent with these results, previous microarray published datasets have identified CD133 as one of the top upregulated genes in GIST, suggesting the *CD133* is part of a 10-gene GIST signature (Nielsen et al., 2002; Segal et al., 2003; Chi et al., 2010). However, our data does not support the prior contention that CD133 expression is associated with an aggressive clinical behavior. Furthermore, from the mRNA signature profile of functional classes of murine ICC, both CD133 and CD44 are overexpressed in small bowel myenteric ICC compared to the tunica muscularis (Chen et al., 2007). More recently, we have shown that *ETV1* is a lineage survival factor that cooperates with KIT in GIST (Chi et al., 2010). Thus *ETV1* knockdown experiments showed that *CD133* was the top downregulated gene, in keeping with a GIST lineage (Chi et al., 2010).

Our *in vitro* experiments show that CD133⁻/CD44⁺ cells have a more aggressive behavior compared to CD133⁺/CD44⁺ and CD133⁻/CD44⁻ cells in aspects of cell proliferation, self-renewal, invasion and SP cells enrichment. These results do not support the hypothesis that CD133⁺ represents a marker for cancer-initiating cells in GIST. Lower expression of CD133 might reflect the emergence of CD133⁻ tumor cells with a clinically more aggressive behavior. Interestingly, downregulation of CD133 in colon cancer has been associated with the acquisition of metastatic potential, pointing to CD133⁻ colon cancer cells as genetically

unstable derivatives of CD133⁺ colon cancer cells with a remarkable ability of forming tumors in immunodeficient mice (LaBarge and Bissell, 2008; Shmelkov et al., 2008).

CD44 is a transmembrane glycoprotein which participates in many cellular process, including growth, survival, differentiation, and motility. In many cell and tissue models, cells adhere to the extracellular matrix hyaluronan via the CD44 glycoprotein (Turley et al., 2002). In cancer, the interaction between hyaluronan and CD44 increases the adhesion, migration and invasion of many cancers (Orian-Rousseau, 2010). In addition, CD44 has also been reported as marker for stem cell isolation and was implicated as a poor prognostic marker in many cancer type (Kaufmann et al., 1995; Ramasami et al., 2000). Similar to CD133, CD44 was also consistently detected in GIST explants and overexpressed in GIST tumors compared to other sarcoma types. The more aggressive functional properties of the CD133⁻/CD44⁺ sorted cell population detected by multiple *in vitro* assays require further investigation.

Based on a method for isolating hematopoietic stem cells (HSC), cells that actively efflux the Hoechst 33342 dye appear as a distinct population of cells at the side of the FACS profile, hence the name “side population” (SP) (Goodell et al., 1996). In human HSC, the SP phenotype is dominated by the expression of the ABCG2 transporter (ATP-binding cassettes [ABC] subfamily G member 2; a.k.a. breast cancer resistance protein, BCRP1). Furthermore, imatinib was shown to be a substrate for the ABCG2 (Burger et al., 2004) and imatinib treatment up-regulates ABC transporters in GIST both *in vivo* and *in vitro* models (Thomas et al., 2004; Rossi et al., 2006), and this could contribute to the development of imatinib resistance. A SP is now recognized in many solid organs and has been isolated from various tumors and thought to contribute significantly to the tumor drug resistance (Hirschmann-Jax et al., 2005). Tumor SP cells not only have the characteristics of tumor stem cells, they are also more resistant to the effects of drugs, and may contribute to the overall drug resistance phenotype of relapsed or resistant cancers. The combined CD133 and Hoechst33342 dye staining in GIST882 cells showed that the SP cells identified are enriched with CD133⁻, a further indication that CD133⁺ expression in GIST is not related to the CSCs.

In summary, our results show that most GISTs have high levels of both CD133 and CD44 by gene expression, in contrast with other types of sarcomas. Furthermore, both flow cytometry and immunofluorescence revealed diffuse CD133 expression in GIST, in keeping with a lineage marker similar with KIT, rather than a true stem cell marker. In addition CD133 was also expressed in the interstitial cells of Cajal (ICC), the cell of origin of GIST. In fact, CD133⁻ cells show functional properties characteristic of a more aggressive behavior. Our findings indicate that CD133 is universally expressed in GIST, with a higher level of mRNA expression in gastric and *KIT*-mutated tumors. *In vitro* results revealed that CD133 negative sorted GIST cells have greater colony formation, higher invasion in matrigel assays, and enrichment in SP cells. All these findings reinforce that CD133, together with other hematopoietic `stem cell markers' consistently overexpressed (i.e. KIT, CD34), represents a lineage marker for GIST.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig.1.

Box-chart representation of mRNA expression of CD133 (A) and CD44 (B) in various soft tissue sarcomas and GIST using the U133A chip. Error bars represents standard error. Ovals on the left hand side of each box represent expression value of individual cases.

Fig. 2.

CD133 expression in GIST samples and ICC: CD133 immuno-fluorescence positive staining in a rectal KIT mutant GIST sample (A, low power, B, inset) and a gastric wild-type pediatric GIST (C); KIT and CD133 dual-staining of ICC hyperplasia in a familial GIST patient with multiple gastric and small bowel tumors and hyperplasia of ICC of the myenteric plexus (D); CD133 (green) and KIT (red) staining of thickened myenteric plexus due to ICC proliferation (E, F); flow cytometry analysis in freshly isolated tumor explants: GIST cells were identified according to the KIT staining, after gating out CD45 positive staining mast cells (G). GIST cells (KIT+/CD45-) analyzed showed a 97.5% double positive CD133/CD44 phenotype and a 2% CD133-/CD44+ phenotype (H).

Fig. 3.

Expression of CD133 and CD44 in the GIST882, GIST-T1, GIST48 and GIST62 cell lines. By flow cytometry appreciable CD133 protein expression was detected in GIST882 (A) and GIST-T1 (B), while GIST48 (C) and GIST62 (D) mainly consist of CD133⁻ cells. Notably, almost all the CD133⁺ cells are also CD44⁺. The ratio of CD133⁺ to CD133⁻ cells in these GIST cell lines remains constant in different confluence conditions. Immunofluorescence staining of GIST882 cells, expressing KIT (E), CD133 (F) and CD44 (G).

Fig. 4.

Dynamic monitoring of cell proliferation by the RT-CES™ system and functional assays. Cells were seeded at a density of 10000 cells/well in ACEA 16× E-Plates; GIST T1 and GIST48 showed a higher proliferation rate than GIST882 (A). In GIST 882, CD133⁺/CD44⁺ sorted cells grew faster than CD133⁺/CD44⁻ cells. Each line represents 8–16 samples. (C) Imatinib response was monitored dynamically by the RT-CES system. The CD133⁻ sorted cells was slightly less responsive to imatinib than CD133⁺ cells (C). Box charts of colony formation, with Y scale showing the number of colony per 100 cells/per well in a 96-well plate; GIST T1 shows the greatest colony formation ability among the three cell lines (D). In GIST882, CD133⁻ cells form more colonies than CD133⁺ cells (E), as well as showing more invasive properties in matrigel invasion assays (F).

Fig. 5.

Identification of side population (SP, thin arrows) and non-SP (thick arrows) in GIST882. Combined staining of CD133 and Hoechst33342 show SP cells are mainly identified within CD133⁻ cells (A), rather than CD133⁺ cells (B). Re-analysis of SP after five passages of culture after sorting shows a prominent side population in CD133⁻ sorted cells (C), compared to CD133⁺ cells (D).

Table 1

Correlation of CD133 and CD44 mRNA expression on U133A chip with various clinicopathological factors in 131 GIST samples

	CD133		CD44	
	(Mean $\bar{x} \pm SE$)	P-value	(Mean $\bar{x} \pm SE$)	P-value
Sites		0.007		0.039
Small bowel (n=57)	10.4 $\pm$ 0.2		10.0 $\pm$ 0.1	
Stomach (n=42)	11.1 $\pm$ 0.3		10.5 $\pm$ 0.1	
Others (n=30)	11.4 $\pm$ 0.3		9.9 $\pm$ 0.2	
Primary/Metastasis		0.069		0.015
Primary(n=45)	11.0 $\pm$ 0.2		10.3 $\pm$ 0.1	
Metastasis(n=22)	10.2 $\pm$ 0.2		9.3 $\pm$ 0.1	
Mutation		0.39		0.0015
WT(n=8)	10.9 $\pm$ 0.3		10.3 $\pm$ 0.1	
EX11 (n=23)	10.9 $\pm$ 0.2		10.0 $\pm$ 0.1	
EX9 (n=3)	11.2 $\pm$ 0.3		10.1 $\pm$ 0.1	
PDGFRA (n=8)	9.9 $\pm$ 0.6		9.8 $\pm$ 0.1	
Age group		0.80		<0.0001
Adult (n=106)	10.9 $\pm$ 0.1		10.0 $\pm$ 0.08	
Pediatric (n=21)	10.84 $\pm$ 0.3		10.9 $\pm$ 0.2	
Young Adult (n=4)	11.4 $\pm$ 0.7		11.0 $\pm$ 0.3	
Imatinib response		0.018		0.10
Resistant (n=43)	10.9 $\pm$ 0.3		10.1 $\pm$ 0.1	
Responsive (n=15)	9.6 $\pm$ 0.4		9.9 $\pm$ 0.1	
Naïve (n=73)	11.0 $\pm$ 0.1		10.37 $\pm$ 0.1	
Mitotic rate *		0.12		0.10
1 (n=21)	10.8 $\pm$ 0.4		10.7 $\pm$ 0.2	
2 (n=20)	11.6 $\pm$ 0.3		10.2 $\pm$ 0.1	

$\bar{x}$ normalized expression value (log2); SE, standard error;

* mitotic rate was analyzed only in primary tumors