Expression study of receptor tyrosine kinase target of Imatinib Mesylate in skull base chordomas

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Abstract

Chordomas are rare neoplasm arising along the axial skeleton. Up to now, the most suitable therapeutic approach is based on a combination of surgical excision and radiotherapy. Chemotherapy in not applied due to its reported low efficacy. Recently, evidence on efficacy of Imatinib mesylate in two patients has been reported. We analyzed 14 chordoma samples for the expression of Imatinib mesylate targets by means of RT-PCR and immunohistochemistry and found that PDGFRα and PDGFRβ are in some cases expressed in neoplastic cells, while the stromal counterpart of the same tumor shows the above receptors. Findings on *PDGFA*/*PDGFB* expression suggest a receptor activated status. Our work provides new insights on the specific localization of Imatinib mesylate targets in skull base chordomas that could be taken in account for the setting up of a pharmacological treatment of this tumor.

Introduction

Chordomas are uncommon slowly growing neoplasms of the axial skeleton with two main sites of origin, the sacrococcigeal region and the skull base (clivus). Treatment of chordomas is still controversial. Surgical excision followed by radiotherapy, in particular proton beam irradiation, seems to be related with the best tumor progression-free survival (1). Chemotherapy is not currently applied in clinical practice because of the described low efficacy(2-5). The course of the disease is often unfavorable because of local recurrence and eventually death. Recently Casali and his colleagues reported the compassionate use of Imatinib mesylate on 6 patients affected by chordoma (1 skull base and 5 sacral chordomas), 4 of whom expressing PDGFRβ (6). Authors observed tumor colliquation in one patient and a decrease in tumor density in another one. The study revealed an antitumor activity of Imatinib mesylate. As this drug exerts its activity by inactivating PDGFRβ, PDGFRα and c-kit, it appears important to investigate their expression in a higher number of chordomas. We therefore evaluated the expression of *PDGFRA*, *PDGFRB* and *KIT* transcripts and proteins and the mRNA expression of their ligands *PDGFA*, *PDGFB* and *SCF* in 14 skull base chordoma (SBC) samples.

Materials and Methods

Tumor samples

The study was performed on 13 skull base chordoma samples and on a recurrence from one of them. Each patient underwent surgery at the Department of Neurosurgery of the San Raffaele Scientific Institute in Milan during the period from August 1997 to December 2005.

Nine patients were male and four were female; the age ranged from 25 to 67 years (average 40,5 years). All the patients had been treated with radiotherapy.

All patients gave informed consent to the study.

RT-PCR analysis

The expression analysis of Imatinib targets was performed by RT-PCR. RNA, isolated from 14 fresh or frozen chordoma samples, was carried out by means of Trizol reagent (Invitrogen, Carlsbad, California); 1µg of total RNA was reverse transcribed using ThermoScript RT-PCR System and oligo-dT primers for first-strand cDNA synthesis according to the instructions of the manufacturer (Invitrogen, Carlsbad, California). Specific primers for *KIT*, *PDGFRA* and *PDGFRB* were designed on different exons for each amplimer (Table I); the ligand primers were synthesized according to Matei (7). cDNAs were analyzed by agarose gel electrophoresis.

Immunohistochemistry

Immunohistochemical analysis was performed on formalin fixed paraffin embedded material. In 12 cases adequate histologic material was available. The following

antibodies were utilized: a rabbit polyclonal PDGF Receptor alpha Antibody (cod.3164, Cell Signaling Technology, Danvers, Massachusetts) diluted 1/100; a rabbit monoclonal PDGF Receptor beta Antibody (cod.3169, Cell Signaling Technology, Danvers, Massachusetts) diluted 1/50; a rabbit polyclonal antibody against c-kit (cod.A4502, Dako, Glostrup, Denmark), diluted 1/500. Briefly the histologic sections were deparaffinized, rehydrated and endogenous peroxidase activity was quenced with 3% Hydrogen peroxide; Tris buffer 0.05M with EDTA 0.01M at pH9 was utilized as antigen retrieval for PDGFRα and PDGFRβ, whereas citrate buffer 0.01M pH 6 was employed for c-kit. The polymer system with DAB as chromogen (Novocastra, Newcastle upon Tyne, United Kingdom) served as detection system.

Results

We performed an expression study of *PDGFRA*, *PDGFRB* and *KIT* genes by RT-PCR and immunohistochemical analysis on 13 primary SBCs and on 1 recurrence from one of them (Figure 1).

The immunohistochemical analysis revealed that the tumor stroma cells were positive for PDGFRα and PDGFRβ expression in all analyzed samples (Figure 2).

In most cases, the neoplastic cells showed focal expression of PDGFRα and PDGFRβ (Figure 2) mainly at the interface with the stromal component, while they were found to be diffusely expressed in 4 and in 3 chordomas respectively. PDGFRα and PDGFRβ were not observed to be expressed in neoplastic cells of 2 and 1 chordomas respectively. c-Kit immunoreactivity was not detected in all but one samples; scattered mast cells served as an internal control in each case.

For the samples where immunohistochemical analysis was not feasible, we report data from the RT-PCR analysis (Figure 1), which shows the transcripts of the three receptors in all samples but one, which lacks *PDGFRB* expression.

We also investigated by RT-PCR analysis the expression of the above TK receptor ligands (*PDGFA*, *PDGFB* and *SCF*), which might prelude the receptor activation by means of an autocrine loop. In particular, *SCF* was found to be expressed only in 1 tumor, while 8 chordomas showed both *PDGFA* and *PDGFB* mRNAs and 1 tumor showed only *PDGFB* mRNA. The results are shown in Figure 1.

Discussion

In the present study we investigate the expression of Imatinib target receptors *PDGFRA*, *PDGFRB* and *KIT* transcripts and proteins, and of their ligand *PDGFA*, *PDGFB* and *SCF* mRNAs in 14 SBCs. The obtained evidence, indicating that all analyzed samples express at least one of the three tyrosine kinase receptors under study and that 9 of them show the ligands *PDGFA* and/or *PDGFB* mRNAs, suggests the activated state of PDGFRs by means of an autocrine/paracrine loop. *SCF* mRNA was detected in one tumor, but the lack of *KIT* expression in the same tumor seems to exclude the biological role of c-kit activation in this instance.

The homodimeric or heterodimeric ligands activate different receptor dimers as shown in Figure 3. It is therefore theoretically possible that 9 chordomas have an activated PDGFRα and/or PDGFRβ.

In fact, the phosphorylated form of PDGFRβ, has been detected in a sacral chordoma by Western Blot analysis, while in the same study the presence of the receptor was postulated in five further samples, after the demonstration of *PDGFB* gene expression (6).

Based on immunohistochemical analysis, we are the first to report the cell-specific localization of PDGFRα and PDGFRβ in SBCs. We observed that both tyrosine kinases are always expressed in the stromal component of the tumor tissue, while the neoplastic cells show a diffuse pattern of expression only in a few tumors. These findings suggest that Imatinib mesylate pharmacological activity might be efficiently exerted in the stroma.

Stromal cells play essential roles in tumor determination: they constitute a solid substrate for tumor growth, are involved in tumor neo-angiogenesis and secrete growth factors (7).

The biological contribution of stroma in the behavior of solid tumors and the significant stromal component in chordoma pinpoint a putative pharmacological action of Imatinib mesylate against chordoma. The reported colliquation of the spinal chordoma mass in a single case following the administration of Imatinib is consistent with the hypothesis of stromal plot necrosis. Casali and colleagues describe midterm signs of a decrease in tumor density on the CT scan and a change in the signal intensity on the MRI scan even without a noticeable impact on tumor size, which indeed also may increase (6).

This effect of Imatinib on the tumor mass might be dependent on the amount of the stromal component of the tumor and can lead to different consequences according to its localization. Tumor swelling in sacral chordomas usually doesn’t lead to any serious or life-threatening clinical situations. An increase in tumor volume in clival chordomas could lead to the possible onset of new neurological deficits and eventually death for breathing failure due to the worsening of a preexisting brainstem compression. However further observations on additional tumors are necessary to appreciate the effect of the treatment on chordomas.

Therefore the above considerations would suggest that the Imatinib use might be indicated in the treatment of chordomas with specific localizations and carefully considered when a preexisting compression of neurological vital structures has been assessed; furthermore its efficacy might be reduced in chordomas with a low stromal component. The response of the stromal component of chordoma, following the administration of Imatinib mesylate, should be delineated in further studies.

This work provides new insights on localization of Imatinib targets in chordoma and might contribute to address pharmacological and clinical protocols.

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Table I. *KIT*, *PDGFRA* and *PDGFRB* primer sequences and PCR-conditions

GENE	Primer Forward	Primer Reverse	PCR conditions	
KIT	GATCCCATCGCAGCTAC	TCATATAGATCCACTGCTG	95°-62°-72° 30"-30"-30"	10 cicles
			95°-56°-72° 30"-30"-30"	35 cicles
PDGFRA	GGGGAAACGATTGTGGTCACC	CCCGCACCTCTACAACAAAAT	95°-62°-72° 30"-30"-30"	40 cicles
PDGFRB	GATTCTGATGCCTACTATGTC	CAGGGTGCGGTTGTCTTTGA	95°-62°-72° 30"-30"-25"	5 cicles
			95°-58°-72° 30"-30"-30"	35 cicles

Figure Legends

Figure 1. Expression of c-kit, PDGFRα, PDGFRβ and their ligands SCF, PDGFA and PDGFB and in 15 chordoma samples by immunohistochemical analysis. +, positive. -, negative. f, focal expression. * data obtained by RT-PCR.

Figure 2. Immunohistochemical analysis showing (A) PDGFRα expression in stromal and tumor cells of chordoma sample 12; (B) PDGFRα immunoreactivity limited to the stromal component of chordoma sample 49; (C) PDGFRβ expression in stromal and tumor cells chordoma sample 20; (D) PDGFRβ is present mainly in the stromal cells with a more limited expression in tumor cell chordoma sample 21. In A, C and D, the original magnification is 400x, in B 200x. Open arrow indicates the neoplastic cells, black arrow indicates stromal cells.

Figure 3. Schematic representation of PDGFR$\alpha\alpha$ PDGFR$\alpha\beta$ and PDGFR$\beta\beta$ activation mechanism upon binding with their ligands PDGFAA, PDGFAB and PDGFBB. Black dots indicate the activated status of receptors.

Figure 1

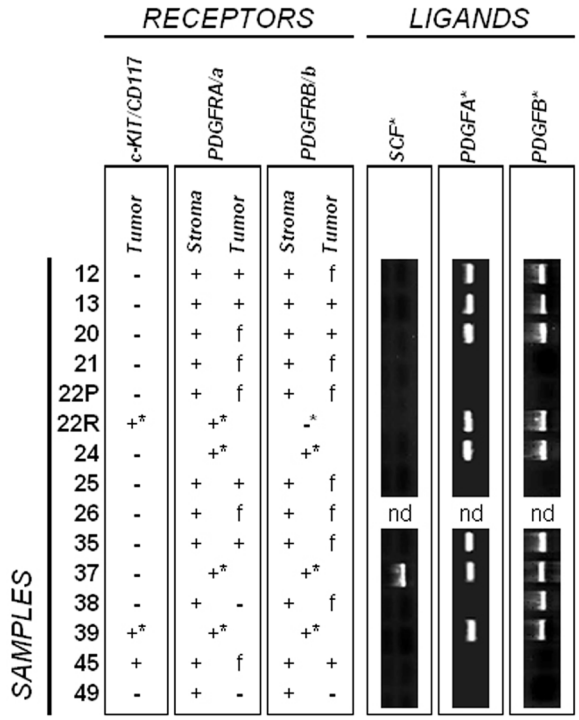
RECEPTORS
LIGANDS
c-KIT/CD117
PDGFRA/a
PDGFRB/b
SCF*
PDGFA*
PDGFB*
Tumor
Stroma
Tumor
Stroma
Tumor
SAMPLES
12
13
20
21
22P
22R
24
25
26
35
37
38
39
45
49
nd
nd
nd

Figure 2

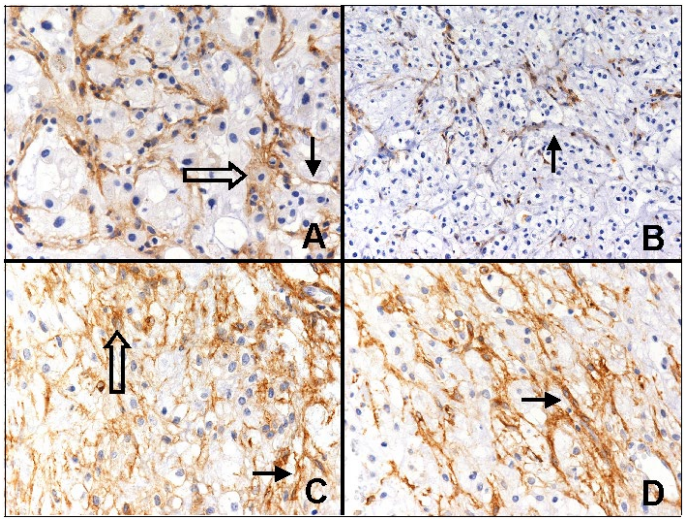

Figure 3