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Review

Insulin-like Growth Factor (IGF) system and gastrointestinal stromal tumours (GIST): present and future

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Summary. In the last decades, the concept that Insulin-like Growth Factor (IGF) axis plays a key role in several steps of tumorigenesis, cancer growth and metastasis has been widely documented. The aberration of the IGF system has been described in many kinds of tumours, providing several lines of evidence in support of IGF receptor type 1 (IGF1R) as molecular target in cancer treatment.

Gastrointestinal stromal tumors (GIST) are the most common mesenchymal tumor of the gastrointestinal tract, commonly characterized in most cases by KIT and PDGFRA gain mutations.

Beyond to the well recognized KIT and PDGFRA gain mutations, in the last years other molecular aberrations have been investigated.

Recently, several lines of evidence about the involvement of the IGF system in GIST have been accumulated.

The aim of this review is to report all current data about the IGF system involvement in GIST, focusing on the current clinical implication and future perspectives.

Key words: Gastrointestinal stromal tumors (GIST), Insulin growth factor receptor 1 (IGF1R), KIT/PDGFRA, Wild-type, Succinate dehydrogenase (SDH).

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Introduction

The Insulin-like Growth Factor (IGF) signalling system, composed of the IGF-receptor type 1 (IGF1R), the insuline receptor (IR), two ligands (IGF1 and IGF2), and six regulatory IGF-binding proteins (IGFBPs), is physiologically involved in the regulation of normal tissue growth and metabolism (LeRoith and Roberts, 2003). The activation of IGF1R via autophosphorylation after IGF1 and IGF2 binding, promotes cell proliferation and survival by the activation of downstream signalling molecules, such as phosphatidylinositol 3-kinase (PI3K), AKT, mTOR, S6 kinase and mitogen-activated protein kinase (MAPK) (Chitnis, et al., 2008) (Fig. 1). In the last decades, the concept that the IGF axis plays a key role in several steps of tumorigenesis, cancer growth and metastasis has been widely documented both on experimental models and population studies (Pollak, 2008; Samani et al., 2007; Seccareccia and Brodt, 2012).

The aberration of the IGF system, even if the molecular mechanisms behind it are still not well clarified, has been described in many kinds of tumours, and several lines of evidence have been provided in support of IGF1R as a molecular target in cancer treatment (Gualberto and Pollak, 2009).

Gastrointestinal stromal tumors (GIST) are the most common mesenchymal tumor of the gastrointestinal tract, commonly characterized in most cases by KIT and PDGFRA gain mutations.

Beyond to the well recognized KIT and PDGFRA gain mutations, in the last years other molecular aberrations have been investigated, especially in that

subset of GIST wild-type (WT) for known driver mutations, defined as KIT/PDGFRA WT GIST (Agaram et al., 2008a; Astolfi et al., 2010; Pantaleo et al., 2011, 2013).

The evidence about the involvement of IGF system in GIST recently accumulated could be separated into four main fields of interest (Fig. 2):

- IGF1R over-expression (Prakash et al., 2005; Agaram et al., 2008b; Tarn et al., 2008; Pantaleo et al., 2009, 2010; Janeway et al., 2010; Italiano et al., 2012; Chou et al., 2012; Belinsky et al., 2013; Lasota et al., 2013; Nannini et al., 2013);
- the correlation between IGF system deregulation and survival (Braconi et al., 2008; Rikhof et al., 2009; Kwon et al., 2012);
- the correlation between IGF system and response to imatinib treatment (Trent et al., 2006; Dupart et al., 2009; Valadao et al., 2012);
- the non-islet cell tumour-induced hypoglycemia in GIST patients (Beckers et al., 2003; Pink et al., 2005; Rikhof et al., 2005, 2009; Hamberg et al., 2006; Singh et al., 2006; Escobar et al., 2007; Davda and Seddon, 2007; Tan et al., 2011).

The aim of this review is to report all current data about the IGF system involvement in GIST, focusing on the current clinical implications and future perspectives.

IGF1R over-expression in GIST

The evidence of IGF1R over-expression in GIST was firstly reported by Prakash et al. in 2005, by a gene expression analysis performed on 7 tumor samples from two children and two young adults, and compared to 10 gastric GIST from adults (Prakash et al., 2005). By an unsupervised analysis all samples clustered within the pediatric and young adult group 385 differentially expressed genes were found between the two groups (Prakash et al., 2005). Among them, IGF1R was up-regulated in the pediatric and young adult samples. Similarly, the gene expression analysis performed on 8 gastric KIT/PDGFRA WT GIST from pediatric patients, compared with 19 gastric GIST from adult patients (12 KIT-mutated, 4 PDGFRA-mutated and 3 KIT/PDGFRA WT), confirmed the distinct gene signature between the two groups with 814 differentially expressed genes, including IGF1R, which was highly over-expressed in pediatric GIST (Agaram et al., 2008b).

Subsequently, Tarn et al. (2008) confirmed these preliminary data in a set of 17 fresh-frozen adult and pediatric GIST samples (14 KIT-mutated, 1 PDGFRA-mutated and 2 KIT/PDGFRA WT) by western blot (WB) analysis, showing that IGF1R was expressed and activated in all GIST but was markedly over-expressed in the two KIT/PDGFRA WT GIST compared with the 15 mutant KIT/PDGFRA ones (Tarn et al., 2008). In this series, the overall IGF1R levels did not correlate with phospho-IGF1R levels, no mutations in IGF1R were found and only in some cases IGF1R over-expression was associated with gene amplification by single nucleotide polymorphisms (SNPs) and fluorescence in situ hybridazation (FISH) analyses (Tarn et al., 2008). Moreover, by immunohistochemical (IHC) analysis, most of the 16 mutant GIST samples showed low or no detectable levels of IGF1R, whereas all of the WT GIST,

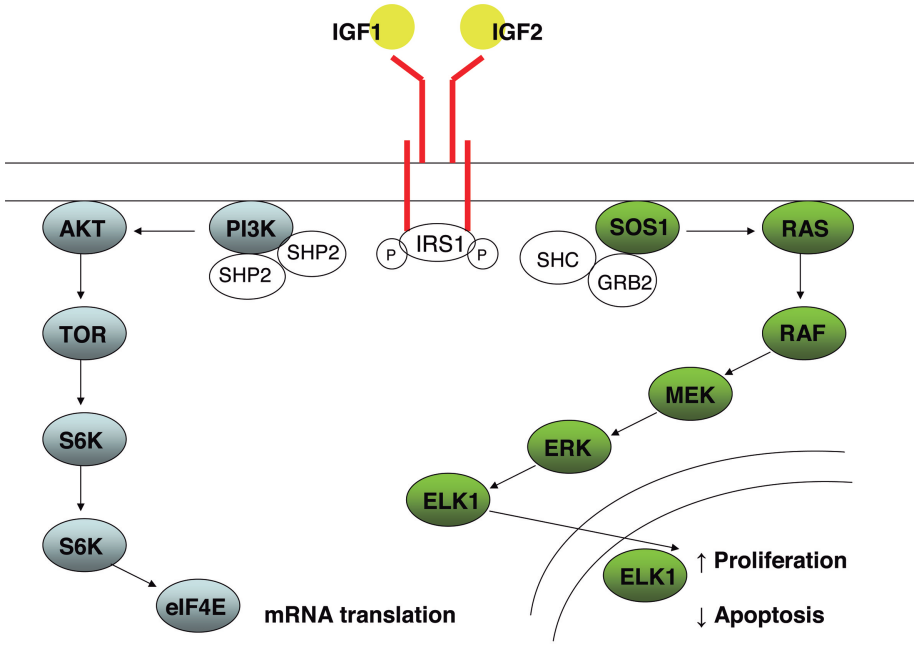

Fig. 1. Insulin-like growth factor downstream pathway.

including the pediatric one, showed strong IGF1R staining (Tarn et al., 2008). In a set of 8 patients with gastric GIST, only the two KIT/PDGFRA WT samples displayed an over-expression of IGF1R by gene expression profiling and IGF1R up-regulation by WB and IHC analyses, without IGF1R amplification by SNPs array analysis (Pantaleo et al., 2009). Similarly, high levels of IGF1R expression were detected in 8 of 9 pediatric KIT/PDGFRA WT GIST, while all 5 KIT-mutant GIST lacked IGF1R expression (Janeway et al., 2010). By SNPs analysis, none of the pediatric KIT/PDGFRA WT GIST had IGF1R amplification (Janeway et al., 2010). More recently, Italiano et al has investigated the expression profiling of all genes encoding the main components of the IGF-signalling pathway in 131 GIST (106 adult, 21 pediatric and 4 young adult) (Italiano et al., 2012). In agreement with previous data, the expression of IGF1R gene was significantly higher in the pediatric group than in the adult group and it was confirmed by quantitative real-time PCR (Italiano et al., 2012). Also in this series, no copy number alterations were detected by FISH analysis, and no mutations of the IGF1R gene were found (Italiano et al., 2012).

On the basis of previously reported data, it is now certain that IGF1R over-expression is not a molecular hallmark of all GIST, but a feature of a distinct subset of KIT/PDGFRA WT GIST, pediatric and of young adults, which share some pathological and clinical features, suggesting the potential role of IGF1R as novel therapeutic target for this subgroup of patients (Pantaleo et al., 2010).

Finally, it is recently emerging that IGF1R over-expression and the loss of function of succinate dehydrogenase (SDH) complex seem to be strictly linked each other (Chou et al., 2012; Belinsky et al., 2013; Lasota et al., 2013; Nannini et al., 2013). Chou et al. firstly assessed SDH subunit B (SDHB) and IGF1R expression by IHC in 8 known SDH-deficient GIST, 3 KIT/PDGFRA WT GIST in the contest of neuro-fibromatosis type 1 (NF1) syndrome, and 40 unselected GIST (5 KIT/PDGFRA WT GIST and 35 KIT/PDGFRA mutated GIST) (Chou et al., 2012). In this series, IGF1R over-expression was found in all eight KIT/PDGFRA WT SDH-deficient GIST and in the two KIT/PDGFRA WT unselected GIST that were SDHB negative at IHC analysis (Chou et al., 2012). On the contrary, all of the 3 KIT/PDGFRA WT GIST NF1-related and the other 38 unselected GIST, which were SDHB-positive, were IGF1R negative (Chou et al., 2012). The status of SDH complex at the genomic and protein level in relation to IGF1R expression at the mRNA and protein level has been assessed in a small set of 7 KIT/PDGFRA WT sporadic GIST patients (Nannini et al., 2013). Likewise, the up-regulation of IGF1R mRNA and an over-expression of IGF1R by WB analysis has been found in all 4 KIT/PDGFRA WT GIST patients harbouring inactivating nonsense or missense SDHA mutations or displaying a SDH dysfunction, compared with either KIT/PDGFRA mutant or KIT/PDGFRA WT GIST without SDH mutations (Nannini et al., 2013). Similarly, an over-expression of IGF1R protein was detected in 11 of 12 KIT/PDGFRA WT SDHB-negative GIST, and 5 of them presented a biallelic inactivation of the SDHA gene, due to germline point mutations accompanied by somatic SDHA allelic losses (Belinsky et al., 2013). This relationship has been recently confirmed in a large series of 1078 well-characterized GIST: IGF1R over-expression was detected in 71/80 of SDH-negative gastric GIST, whereas none of the 373 intestinal GIST were IGF1R positive, suggesting that firstly IGF1R over-expression could be a hallmark of SDH-deficient GIST as a group, rather than of pediatric or KIT/PDGFRA WT GIST *per se*, and that secondly, IGF1R IHC positivity may also be considered a useful surrogate marker to identify SDH-deficient GIST

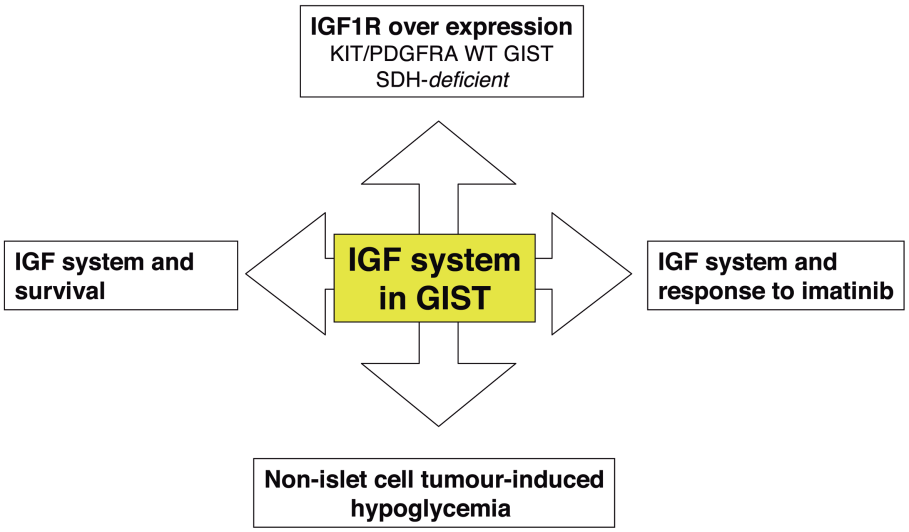

Fig. 2. The involvement of IGF system in GIST.

(Lasota et al., 2013).

IGF system and survival in GIST

Braconi et al. evaluated the IGF1 and IGF2 IHC status in 94 GIST patients (59 exon 11 KIT-mutated, 10 exon 9 KIT-mutated, 1 exon 17 KIT-mutated, 11 exons 12 and 18 PDGFRA-mutated, 13 KIT/PDGFRA WT) (Braconi et al., 2008). According to staining intensity and extension of cytoplasmic expression, IGF1 and IGF2 expression was absent in 25 and 48 cases, moderate in 29 and 16 cases and strong in 40 and 30 cases, respectively (Braconi et al., 2008). Of note, IGF1 and IGF2 expression were strictly correlated with the class of risk: in particular a strong IGF expression was found among GIST with higher mitotic index, larger size, metastatic onset of the disease and disease relapse (Braconi et al., 2008). Moreover, a significant worsening of the disease-free survival (DFS) with the increase of IGF1 and IGF2 expression was found (Braconi et al., 2008). The correlation between the different degrees of IGF expression and the different prognosis suggests the potential role of IGF1 and IGF2 as a new prognostic molecular factor useful to predict relapse and better stratify GIST with an aggressive behaviour.

In order to define the clinical relevance of IGF-related proteins in GIST, Rikhof et al. have determined the plasma levels of IGF1, total IGF2, pro-IGF2E, IGF-binding protein (IGFBP) -2, IGFBP3 and IGFBP6 in a cohort of 22 GIST patients, before and during treatment with imatinib (Rikhof et al., 2009). Patients with larger tumour size and metastatic disease presented the highest pro-IGF2E plasma levels, although elevated plasma levels of any component of the IGF system investigated did not predict progression-free survival (PFS) (Rikhof et al., 2009). Only the plasma levels of IGFBP-2 were higher in patients with progressive disease in comparison with patients who responded to imatinib, even if the eventual role of IGFBP-2 as a laboratory marker for disease progression in GIST remains to be defined, because the levels of IGFBP-2 may be influenced by the nutritional status (Rikhof et al., 2009).

More recently, the determination of four IGF-1 (+2995C/A, +533C/T, IVS2-16540A/G, Ex4-177G/C) and one IGF-2 (IVS1+1280A/G) gene polymorphisms failed to define their impact on the prognosis in 213 consecutive Korean patients with surgically resected GIST (Kwon et al., 2012). In fact, in a multivariate analysis including age, gender, primary tumour site, mitotic index and risk stratification, no significant association was observed between IGF-1 and IGF-2 gene polymorphism and survival (Kwon et al., 2012).

The correlation between IGF system and response to imatinib treatment

Trent et al. studied the early molecular effects of imatinib antitumor activity in GIST by microarray technology, real-time PCR validation and fluoro-deoxyglucose-positron emission tomography (FDG-PET) imaging (Trent et al., 2006). They found that 55 genes were altered after exposure to imatinib at both 24 and 48 hours only in imatinib-sensitive GIST882 in comparison with imatinib-resistant sarcoma cell lines

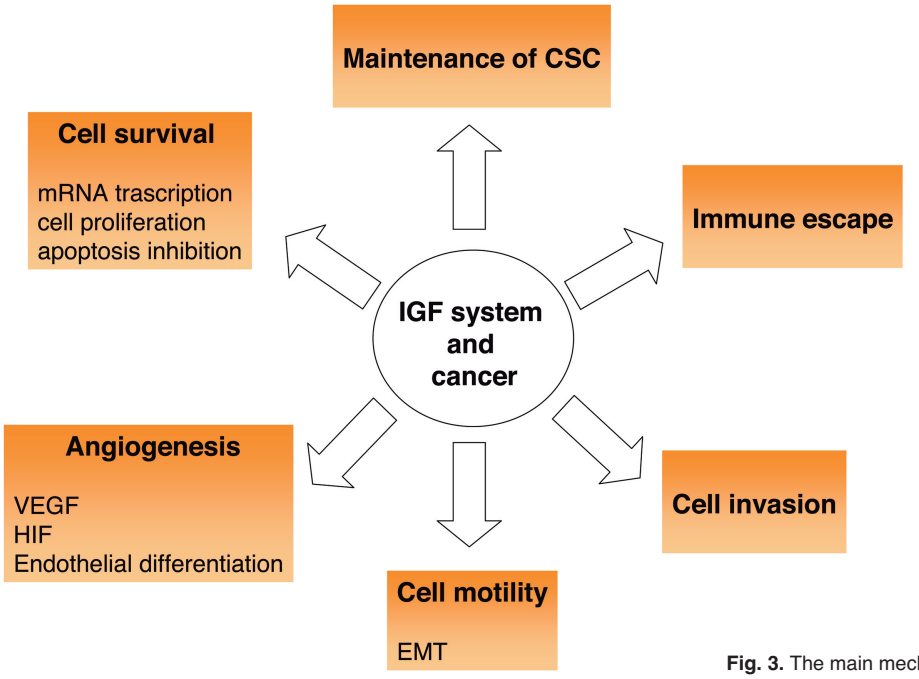

Fig. 3. The main mechanisms of IGF system involvement in cancer.

and imatinib-resistant GIST TM03 cell line (Trent et al., 2006). Among these genes, IGFBP-3 was up-regulated only in GIST882 cells by WB analysis (Trent et al., 2006). Moreover, up to a 7-fold induction of IGFBP-3 mRNA was found in tumour samples from GIST patients with low residual FDG uptake, whereas there was an up to 12-fold reduction in IGFBP-3 in those patients with high residual FDG uptake after imatinib treatment, suggesting that IGFBP-3 may be a important early molecular marker of antitumor activity of imatinib in GIST (Trent et al., 2006).

Subsequently, the same authors tried to determine whether IGFBP-3 directly mediates the cytotoxicity of imatinib in GIST cells, by manipulating IGFBP-3 levels in two imatinib-sensitive cell lines (GIST882 and GIST-T1) and observing cell viability after treatment (Dupart et al., 2009). They found that IGFBP-3 down-regulation in GIST882 cells resulted in a loss of cell viability and partial resistance to imatinib, whereas IGFBP-3 over-expression did not enhance or abrogate the cytotoxic effects of imatinib in GIST-T1 cells, demonstrating that IGFBP-3 may have cell-dependent effects on GIST cell viability and in mediating imatinib response (Dupart et al., 2009).

More recently, Valadao et al. investigated the role of IGF1R in GIST pathogenesis as potential biomarkers for prediction of response to imatinib treatment in 76 metastatic GIST patients (51 KIT-mutated GIST, 5 PDGFRA-mutated GIST and 4 KIT/PDGFRA WT) (Valadao et al., 2012). In contrast with most of the data reported in the literature, they found that IGF1R over-expression by IHC analysis did not correlate with mutations status and observed a statistically significant association between IGF1R expression and type of response to imatinib (Valadao et al., 2012). In particular, the higher IGF1R expression was found to be associated with a lower objective response rate to imatinib, without affecting the PFS and OS (Valadao et al., 2012).

The non-islet cell tumour-induced hypoglycemia in GIST patients

The non-islet cell tumour-induced hypoglycemia (NICTH) in GIST patients has been reported in several single clinical cases (Beckers et al., 2003; Pink et al., 2005; Rikhof et al., 2005, 2009; Hamberg et al., 2006; Singh et al., 2006; Davda and Seddon, 2007; Escobar et al., 2007; Tan et al., 2011). As is already known, NICTH is considered a paraneoplastic phenomenon caused by excessive autocrine production of 'big'-IGF-II (Frystyk et al., 1998). Biochemically, NICTH is characterised by hypoglycaemia, low levels of insulin and c-peptide and an IGF-II: IGF-I ratio of 10 or more (LeRoith. 1999). Moreover, it has been shown that 'big'-IGF-II may also stimulate tumour growth via autocrine and paracrine loops, suggesting a correlation between the severity of hypoglycemia, the tumour burden and likely the disease progression (Daughaday, 1990; Renehan et al., 2000; Baron-Hay et al., 2004; Vorwerk et al., 2005). In this regard, as mentioned before, a higher amount of plasma levels of IGFBP-2 were found also in GIST patients with progressive disease in comparison with patients who responded to imatinib (Rikhof et al., 2009). Similarly, all single clinical cases reported in literature have described the onset of severe episodes of NICTH in patients with large metastatic and/or progressive GIST, suggesting a potential role of IGFII in clinical practice as a marker to identify patients with higher risk for the development of hypoglycemia, as well as a predictive marker of disease progression, even if the latter remains to be established (Beckers et al., 2003; Rikhof et al., 2005; Pink et al., 2005; Hamberg et al., 2006; Singh et al., 2006; Escobar et al., 2007; Davda and Seddon, 2007; Tan et al., 2011).

Discussion

It is now a fact that the IGF axis plays a key role in several steps of tumorigenesis processes, from the initial malignant transformation to tumour progression and metastases development, by several molecular mechanisms (Samani et al., 2007; Pollak, 2008; Seccareccia and Brodt, 2012) (Fig. 3). It is well known that the activation of IGF1R, via autophosphorylation after IGF1 and IGF2 binding, promotes cell proliferation and survival by the activation of PI3K, AKT, mTOR and MAPK downstream signalling pathways (Chitnis et al., 2008). Moreover, other mechanisms have been recently identified, supporting even more the relationship between the IGF system and cancer (Stoeltzing et al., 2003; Zhang et al., 2003; Menu et al., 2004; Treins et al., 2005; Graham et al., 2008; Huang et al., 2009; Metalli et al., 2010; Nakamura et al., 2010; Walsh and Damjanovski, 2011; Durfort et al., 2012; Piecewicz et al., 2012; Seccareccia and Brodt, 2012). Firstly, the IGF system seems to be involved in tumour angiogenesis by regulating the expression of vascular endothelial growth factor (VEGF) via nuclear translocation of hypoxic inducible factor (HIF), or via MAP, MEK/ERK or AKT pathways depending on the cell type (Stoeltzing et al., 2003; Menu et al., 2004; Treins et al., 2005). Furthermore it has been suggested that IGF-1 can also promote neovascularisation directly and inducing the endothelial differentiation of embryonic stem cells (Nakamura et al., 2010; Piecewicz et al., 2012). Secondly, the IGF system may promote tumour cell motility, by affecting the epithelial-mesenchymal transition (EMT) via the activation of transforming growth factor (TGF)-beta and of transcription factors of the Snail family, and also interacting with integrins (Graham et al., 2008; Metalli et al., 2010; Walsh and Damjanovski, 2011). It also promotes tumour cell detachment, motility ad invasion by inducing the expression of matrix metalloproteinases (MMPs) (Zhang et al., 2003). Thirdly, in secondary sites, the IGF system can also promote tumour cell survival by modulating the anti-tumour immune response (Durfort et al., 2012). Finally some evidence suggest that the IGF system also contributes to the development and maintenance of

cancer stem cell (CSCs), promoting the selection of resistant clones and thus favouring the metastatic dissemination (Bendall et al., 2007; Huang et al., 2009).

The signalling pathways of the IGF seem to be implicated in the pathogenesis of a wide variety of epithelial and mesenchymal neoplasms and in most cases the deregulation of the IGF system is due to the over-expression of the IGF-1R or the establishment of signalling loops via the autocrine or paracrine production of IGF1 and IGF-2 (Scotlandi and Picci, 2008; Scagliotti and Novello, 2012; Bruchim and Werner, 2013). All these findings above have provided consistent evidence in support of considering IGF1R as a target in cancer therapy, and several strategies aimed at the inhibition of the IGF signalling pathway, such as monoclonal antibodies or small tyrosin kinase inhibitors anti-IGF1R, have been recently developed (Yee, 2012).

In the last years, some lines of evidence about the involvement of IGF system also in GIST have been accumulated (Beckers et al., 2003; Pink et al., 2005; Prakash et al., 2005; Rikhof et al., 2005, 2009; Hamberg et al., 2006; Singh et al., 2006; Trent et al., 2006; Davda and Seddon, 2007; Escobar et al., 2007; Tan et al., 2011; Agaram et al., 2008b; Braconi et al., 2008; Tarn et al., 2008; Dupart et al., 2009; Pantaleo et al., 2009, 2010; Janeway et al., 2010; Chou et al., 2012; Italiano et al., 2012; Valadao et al., 2012; Belinsky et al., 2013; Lasota et al., 2013; Nannini et al., 2013). Among them, the most intriguing data are those about the findings of IGF1R over-expression in a specific subtype of GIST, having common clinical and molecular hallmarks. In particular, the IGF1R over-expression seems to be limited to pediatric and young-adult GIST, who sharing some pathological and clinical features: the lack of KIT and PDGFRA mutations, the female prevalence, the gastric primary localization, the multifocal presentation, and most frequent lymph nodal involvement and the indolent course of disease even if metastatic (Prakash et al., 2005; Agaram et al., 2008b; Tarn et al., 2008; Pantaleo et al., 2009, 2010; Janeway et al., 2010; Italiano et al., 2012).

In addition, KIT/PDGFRA WT GIST have a gene signature profoundly different from both mutated-GIST and murine mature interstitial cells of Cajal (ICCs), especially in the expression of those genes belonging to neural tissues as well as in the expression of IGF1R (Pantaleo et al., 2011).

Of note, the precursors identified by Lorincz et al. are characterized by an IGF1R-positive, $KIT^{(low)}$, $CD44^{(+)}$, $Insr^{(+)}$ phenotype and their differentiation into mature ICCs, which are phenotypically IGF1R-negative, seems to depend on IGF1 (Lorincz et al., 2008). Moreover, it has been postulated that the IGF1R pathway may be essential for structural and functional maintenance of the ICC network and the tumour development may be initiated when the IGF1R-mediated balance between cell renewal and differentiation is altered (Huizinga and White, 2008). Therefore, firstly, the IGF1R pathway may play a key role in the development of KIT/PDGFRA WT GIST (Pantaleo et al., 2011). Secondly, given that KIT/PDGFRA WT GIST and the precursors of ICCs have in common the expression of IGF1R, whereas mature ICCs and mutated GIST have a more overlapped gene expression profile, it could be supposed that KIT/PDGFRA WT GIST may derive from ICCs in a different differentiation step, of which IGF1R expression is the main molecular hallmark (Pantaleo et al., 2011).

More recently, IGF1R over-expression has been further limited to SDH-deficient GIST, which apart from the previous clinical and molecular features have in common the loss of SDH complex, as seen by immunohistochemical loss of SDHB expression, due in most cases to germline and/or de novo mutations of SDH complex with prevalence for mutations within SDHA (Chou et al., 2012; Nannini et al., 2013; Belinsky et al., 2013; Lasota et al., 2013; Miettinen et al., 2013; Pantaleo et al., 2013). This latter finding is consistent with the possible biological relationship between the IGF1R pathway and SDH-complex, of likely metabolic origin, suggesting that targeting IGF1R as a novel treatment strategy could be circumscribed only to SDH-deficient GIST. It is probably for this reason that the treatment with a anti-IGF1R fully human monoclonal antibody R1507 in a young-adult patient with NF1-associated KIT7PDGFRA WT GIST, likely SDH positive and IGF1R negative like most NF1-associated GIST, has been ineffective (Day et al., 2011).

Moreover, in clinical practice, the IGF1R could be considered as an additional immunohistochemical marker in the identification of SDH-deficient GIST and in the differential diagnosis between intestinal GIST, which are IGF1R-negative, and primary intestinal sarcomas, including clear cell sarcomas, leiomyo-sarcomas, and undifferentiated sarcomas, which are IGF1R-positive conversely (Miettinen et al., 2013).

All the reviewed data provide consistent evidence in support of the biological relevance of the IGF signalling pathway in a restricted subset of GIST. However, some aspects remain to be clarified. First of all, the mechanism underlying the over-expression of IGF1R in GIST is still unsettled, because no gene amplification and mutations have been identified up to now. This is essential for understanding whether IGF1R can be considered a therapeutic target in GIST or if there is another downstream target biologically more relevant. Secondly, given that the impairment of the IGF pathway seems to be limited to a small subset of GIST, it is essential to define the molecular features of this subgroup, for the better selection of patients who may benefit from anti-IGF1R therapies. It is therefore mandatory that future prospective clinical trials are aimed at the identification of molecular markers predictive of the likelihood of response to anti-IGF1R treatment. Finally, the translation into clinical practice of the IHC status of IGF1R as a diagnostic tool is still far off until it can be validated in a large cohort of patients.

Conflict of interest. The authors have no conflicts of interest to declare

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