Chapter 4
MicroRNAs and Gastrointestinal Stromal Tumor

Pinar Akçakaya and Weng-Onn Lui

Abstract Gastrointestinal stromal tumor (GIST) is the most commonly diagnosed mesenchymal tumor in the gastrointestinal tract. This tumor type is driven by gain-of-function mutations in receptor tyrosine kinases (such as *KIT*, *PDGFRA*, and *BRAF*) or loss-of-function mutations in succinate dehydrogenase complex subunit genes (*SDHx*). Molecular studies on GIST have improved our understanding of the biology of the disease and have led to the use of targeted therapy approach, such as imatinib for *KIT/PDGFRA*-mutated GIST. Recently, microRNAs have emerged as important regulators of *KIT* expression, cancer cell behavior, and imatinib response in GIST. This chapter aims to provide an overview on current understanding of the biological roles of microRNAs in GIST and possible implications in prognosis and therapeutic response.

Keywords microRNA • GIST • Prognosis • Diagnosis • Therapy • Biomarker

Introduction

Gastrointestinal stromal tumors (GISTs) comprise one-fifth of soft tissue sarcomas, making them the most common sarcoma of the gastrointestinal tract [1]. The annual incidence of GIST is between 11 and 19.5 per million [2–5], and it has a prevalence of about 130 cases per million population [2–4]. For many years, GISTs were considered as smooth muscle sarcomas based on their morphology, and had been misdiagnosed as leiomyomas, leiomyosarcomas, or leiomyoblastomas. The prognosis of advanced GIST was very poor due to resistance to conventional chemotherapy and radiotherapy prior to the discovery of targeted therapies [6].

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In the late 1990s, two groundbreaking discoveries had revolutionized the approach to diagnosis and treatment of GIST: (1) majority of GISTs (>95 %) were found immunohistochemically positive for the tyrosine kinase receptor KIT (also known as CD117) [7], and (2) *KIT* gene mutations were identified in 70–80 % of GISTs [8]. To date, KIT immunostaining and mutation screening are used as key diagnostic markers in clinical practice for GISTs, and mutant KIT is a clinically important therapeutic target in GISTs. The evolution of understanding the biology of GIST transformed it from a challenging chemotherapy-resistant disease to a model for molecular targeted therapy.

Although the initial events in GIST development are well characterized, the prognosis is clearly influenced by other genetic or epigenetic events that are still poorly understood. Aberrant microRNA expression is common in a wide range of human cancers. Accumulated evidence has shown that microRNAs are associated with clinical and pathological features in GIST, suggesting their important roles in GIST development.

This chapter gives a brief background on clinical features and biology of GIST, and provides an overview of the current knowledge on involvement of microRNAs in GIST tumorigenesis and therapeutic response.

Gastrointestinal Stromal Tumor

GISTs are thought to originate from the interstitial cells of Cajal (ICC) or their stem-like precursors [7, 8]. ICC function as pacemaker in the gastrointestinal tract that controls peristaltic contractions [7]. GISTs can be found anywhere along the gastrointestinal tract, but predominantly occur in the stomach (50–60 %) and the small intestine (30–35 %), less frequently in the colon/rectum (5 %) and esophagus (<1 %) [9]. These tumors can arise at any age, with a median age of diagnosis at 63 years [1, 9]. The tumor size varies between 2 and 30 cm at the time of diagnosis [10].

Oncogenic Mutations

The main initial event in GIST tumorigenesis is gain-of-function mutations in *KIT* (v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog) or *PDGFRA* (platelet-derived growth factor-α) genes. These genes are located on the long arm of chromosome 4 (4q12), and encode transmembrane proteins that belong to the type III tyrosine kinase receptor family.

Under normal physiological conditions, activation of KIT and PDGFRA receptors is controlled by spatial and temporal expression of their respective ligands, SCF and PDGF. Binding of these ligands to the receptors results in homodimerization, transphosphorylation of the tyrosine residues, and kinase activation that initiates signal

transduction cascades promoting cell proliferation, growth, and survival [11–13]. About 75 % of GISTs harbor *KIT* mutations [14], whereas 10 % of GISTs harbor *PDGFRA* mutations [15, 16]. These mutations disrupt the autoregulatory mechanisms and cause ligand-independent constitutive activation of the encoded tyrosine kinase receptors [17], which results in aberrant cell growth and tumor formation [18]. Activation of KIT or PDGFRA stimulates several downstream signaling pathways such as mitogen-activated protein kinase (MAPK), phosphatidylinositol-3-kinase (PI3K)/AKT/mTOR and signal transducer, and activator of transcription 3 (STAT3) [19–21].

About 10–15 % of GISTs do not harbor *KIT* or *PDGFRA* mutations. These tumors display mutations in multiple cancer genes, including succinate dehydrogenase complex subunit genes (*SDHA, SDHB*, *SDHC*, and *SDHD*) (50 %) [22, 23], *BRAF* V600E substitution (13 %) [24], neurofibromin 1 (*NF1*) (7 %) [25, 26], and RAS family members [27]. Different signaling pathways in GIST are illustrated in Fig. 4.1.

Unlike adult GISTs, pediatric GISTs (1–2 % of all GISTs) are rarely positive for *KIT* or *PDGFRA* mutations, despite expressing KIT at similar levels as adult GISTs [28]. Gene expression pattern of these tumors is also different from adult GISTs [29, 30], suggesting alternative mechanisms of KIT activation or distinct pathways in pediatric GISTs.

Chromosomal Changes in GIST

Cytogenetic studies demonstrated that about 65 % of GISTs have either monosomy of chromosome 14 or partial loss of 14q [31–33]. Loss of heterozygosity and comparative genomic hybridization studies identified two hotspot regions (14q11.2 and 14q32), pointing tumor suppressor genes at these loci might be important for GIST development [32, 34]. Several candidate genes are suggested within these regions, such as *PARP2, APEX1*, and *NDRG2* genes at 14q11.2, *SIVA* [35] and microRNA clusters at 14q32 [36, 37].

Several chromosomal abnormalities have been associated with malignant behavior in GIST. Loss of the long arm of chromosome 22 is observed in approximately 50 % of GISTs and associated with malignancy [31, 33, 38]. Chromosome 9p21 deletion causes inactivation of the tumor suppressor gene *CDKN2A* and associated with metastatic behavior [39–42]. Gains on chromosomes 8q (including *MYC*), 3q (including *SMARCA3*) and 17q are associated with metastasis [32, 43–45].

Treatment of GIST

Surgical resection is the main therapy for localized GIST, with the goal of complete resection and avoidance of tumor rupture [46]. However, surgery is sometimes not applicable for metastatic GISTs or clinically unresectable GISTs. A small molecule

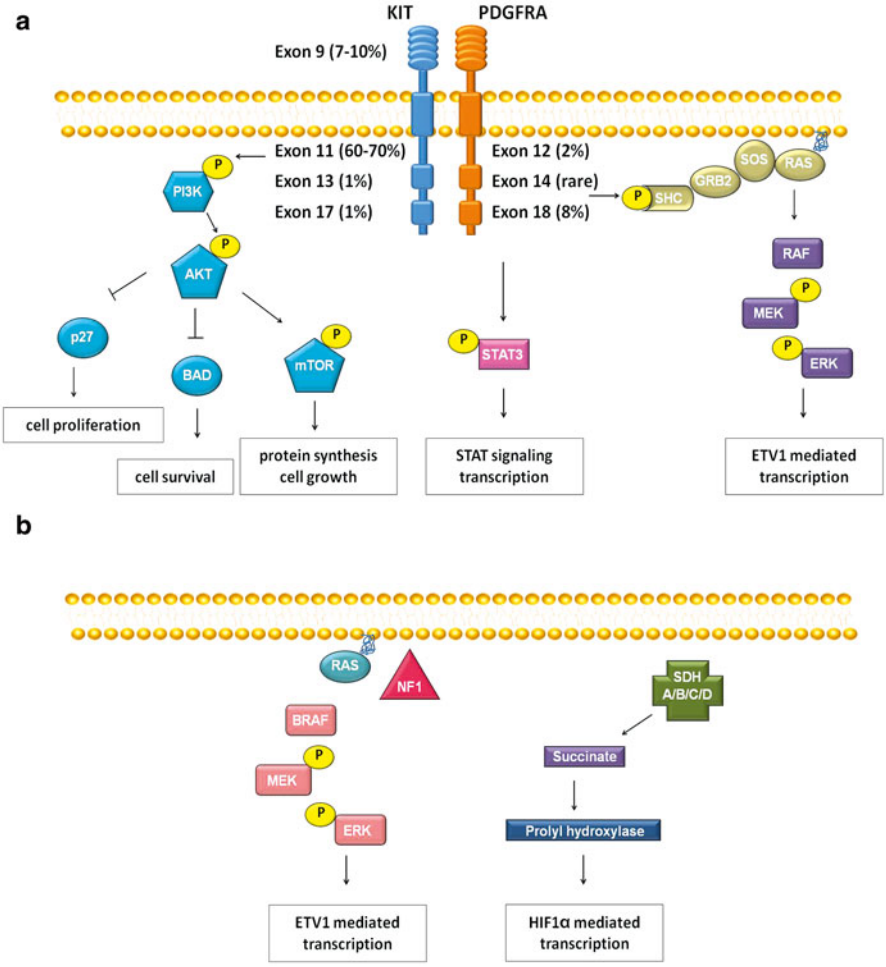

Fig. 4.1 Signaling pathways in GIST. (**a**) KIT and PDGFRA signaling pathways. Mutations in *KIT* or *PDGFRA* activate MAPK, PI3K/AKT/mTOR, and STAT3 pathways. The overall percentage of specific mutation sites is given in parentheses. (**b**) Signaling pathways in "wild-type" GISTs. Mutations in *NF1*, *BRAF*, or *RAS* lead to increased MAPK signaling. Mutations in one of the SDH genes (*SDHA, SDHB, SDHC*, *or SDHD*) lead to succinate accumulation, which inhibits prolyl hydroxylase-mediated HIF1α degradation and thereby increased HIF1α-mediated transcription of *VEGF* and *IGF. P* Phosphate group (Modified from Akcakaya P, thesis for doctoral degree 2015, ISBN 978-91-7549-730-3)

tyrosine kinase receptor inhibitor, such as imatinib mesylate, is used for the treatment of advanced GISTs.

Imatinib can selectively block the enzymatic activity of both transmembrane receptor tyrosine kinases KIT and PDGFRA [47, 48]. It competes with ATP for the ATP-binding pocket located in the kinase domain, and blocks the phosphorylation of the tyrosine kinase receptors. Binding of imatinib inhibits the activation of

downstream survival pathways such as PI3K-mTOR and MAPK [19], and induces cell apoptosis through BIM [49] and soluble histone H2AX [50]. In addition, imatinib reduces the expression of indoleamine 2,3-dioxygenase (IDO) [51], which is an enzyme that produces immunosuppressive metabolites. Reduction of IDO causes depletion of regulatory T cells and increase of tumor-infiltrating $CD8^+$ T cells. Thus, imatinib stimulates an anticancer immune response by diminishing IDO-mediated immunosuppression.

The majority of GIST patients with advanced disease get a clinical benefit from imatinib treatment. Imatinib achieved disease control in 70–85 % of patients with advanced GIST, median progression-free survival increased from 8–10 months to 20–24 months, and median overall survival increased from 18–20 months to 50 months [52–54]. However, resistance to imatinib is one of the biggest obstacles in current GIST clinical practice.

Approximately 10 % of patients progress within 6 months of initial therapy, which is defined as primary resistance to imatinib [53–56]. Primary resistance shows stronger correlation with certain tumor genotypes, such as wild-type *KIT* or *PDGFRA*, *KIT* exon 9 mutations and *PDGFRA* D842V mutation [57–60]. In addition, 50–60 % of the initially responding patients develop disease progression within 2 years, regarded as secondary or acquired resistance [53–56]. The main mechanism of acquired resistance is the acquisition of secondary mutations in the kinase or loop domain of KIT or PDGFRA [61]. Several alternative mechanisms of resistance have been described. Kinase switching is one of them and several kinases have been involved in such mechanism. AXL is an oncogenic tyrosine kinase receptor that regulates the same downstream signaling pathways as KIT. Kinase switching from KIT to AXL was observed in imatinib-resistant GIST cell lines and clinical samples [62]. Besides AXL, a switch from KIT to FAK and FYN activation has also been reported in GIST cells upon acquisition of imatinib resistance, and phosphorylated FAK inhibition can re-sensitize the resistant cells to imatinib-induced cell death [63]. FAK has also been implicated in growth and survival of imatinib-resistant GIST cells [64]. In addition, gene amplification of *KIT* or *PDGFRA* was shown as a potential mechanism leading to either primary or secondary resistance [65]. Moreover, microRNAs have also been shown to play a role in imatinib resistance in GIST, as described in the following section.

MicroRNA Deregulation in GIST

MicroRNA signature of GISTs was first described by Subramanian and colleagues in 2008 [66]. The study compared microRNA profiles of 27 sarcomas with different histological types, and demonstrated that GISTs were clearly distinguished from other sarcomas based on their microRNA expressions (Table 4.1). This distinction implicates the role of microRNAs in GIST tumorigenesis and their potential applications as diagnostic markers or therapeutic targets in GIST. Compared to other sarcoma types, *miR-221–222* and *miR-17–92* clusters were expressed at lower level

Table 4.1 MicroRNA profiling studies in GIST

	MicroRNAs		
Comparison	Up	Down	Ref
GISTs vs. sarcomas	*miR-140*, miR-29c , miR-29b, miR-22, miR-30a-5p, miR-30d, miR-99b, miR-30e-5p, miR-143, miR-29a, miR-30c, miR-145, miR-125a, let-7b, miR-10a*	*miR-368, miR-133b, miR-1, miR-376a, miR-133a, miR-200b, miR-221, miR-222, miR-92*	[66]
GIST vs. GI-LMS	*miR-29c, miR-497, miR-30a, miR-603, miR-330-3p, miR-96, miR-527*	*miR-222, miR-221, miR-382, miR-938, miR-21, miR-21*, miR-155, miR-645, miR-297, miR-190b, miR-20a, miR-18a, miR-17, miR-19a*	[67]
14q − vs. 14q +		14q.32.31 microRNA cluster, e.g.: *miR-134, miR-370*	[37]
		14q.32.33 microRNA cluster, e.g.: *miR-495, miR-376, miR-134, miR-377, miR-539*	[36]
Gastric vs. intestinal	*miR-504, miR-7-1*, miR-598, miR-24-1-5p*	*miR-220c, miR-329, miR-370, miR-210, miR-409-3p, miR-376a, miR-376c*	[37]
	miR-124a, miR-199b, miR-451, miR-663, miR-10a, miR-218, miR-638, miR-24-3p, miR-27b, miR-128b, miR-588, miR-518c, miR-199a, miR-346, miR-200a, miR-526a, miR-625, miR-489, miR-140, miR-23b*	*miR-383, miR-136, miR-146a, miR-409-3p*	[36]
High risk vs. low risk		*miR-377, miR-409-3p, miR-376a*, miR-376b, miR-127, miR-136, miR-214, miR-150-5p, miR-495, miR-154*, miR-497, miR-381, miR-132, miR-195, miR-487b, miR-335, miR-146b, miR-342, miR-363*, miR-100, miR-21, miR-424, miR-487a, miR-16, miR-133b, miR-140, miR-125b, miR-23b, miR-365, miR-30e-5p, miR-152, miR-26b*	[36]
	miR-196a		[69]
		miR-483-5p, miR-1268, miR-508-5p, miR-1915, miR-762, miR-452, miR-371-5p, miR-638, miR-744, miR-1225-5p, miR-1272, miR-137, miR-885-3p, miR-133b, miR-206, miR-1261, miR-939, miR-572, miR-767-3p, miR-1228, miR-892b, miR-589, miR-149*, miR-526b*	[70]

(continued)

Table 4.1 (continued)

	MicroRNAs		
Comparison	Up	Down	Ref
PDGFRA- vs. *KIT*-mutated	*miR-1229, miR-1234, miR-766, miR-652, miR-629, miR-200c, miR-181a-2*, miR-342-3p, miR-151-5p, miR-185, miR-146b-5p, miR-132*, miR-132, miR-150, miR-509-3p*	*miR-330-5p*	[37]
Pediatric vs. adult mutant	*miR-7-2*, miR-15a, miR-16, miR-34c-5p, miR-125a-3p, miR-125b-1*, miR-126, miR-129-3p, miR-186, miR-190, miR-192, miR-210, miR-214, miR-345, miR-361-5p, miR-383, miR-422a, miR-423-5p, miR-450a, miR-450b-5p, miR-488*, miR-488, miR-491-5p, miR-523, miR-542-5p, miR-551b*, miR-576-3p, miR-590-5p, miR-744, miR-873*	*let-7f-2*, miR-139-5p, miR-152, miR-181a-2*, miR-193b*, miR-340, miR-365, miR-455-3p, miR-886-3p, miR-886-5p*	[71]
Imatinib resistant vs. sensitive	*miR-301a, miR-30c, miR-146b-5p, miR-125a-5p, miR-193b, miR-25, let-7d, miR-365, miR-30a-3p, miR-483-3p, let-7e, miR-103, miR-362-5p, miR-15b, miR-151-3p, let-7f, miR-324-5p, miR-30b, let-7a, let-7i, miR-151-5p, miR-130b, miR-660, miR-107, miR-532-5p, miR-214, miR-143*	*miR-574-5p, miR-134, miR-638, miR-762, miR-1224-5p, miR-1225-5p, miR-150-3p, miR-1207-5p, miR-1915, miR-1268, miR-139-3p, miR-663, miR-1249, miR-296-5p, miR-188-5p, miR-939, miR-1228*	[72]
Metastatic vs. nonmetastatic	*miR-301a-3p, miR-30c, miR-25, miR-455-3p, miR-130b, miR-148a, miR-362-5p, miR-660, miR-532-5p, miR-151-3p, miR-214, miR-193b, miR-146b-5p, miR-99b, miR-181a, miR-107, miR-193a-3p, miR-483-3p, miR-24-1-5p*	*miR-134, miR-939, miR-762, miR-1914-3p, miR-1290, miR-1305, miR-1202, miR-1207-5p, miR-1225-5p, miR-663, miR-575, miR-150-3p, miR-125a-3p, miR-1246, miR-1308, miR-139-3p, miR-188-5p, miR-1268, miR-1224-5p, miR-638, miR-150-5p, miR-1915, miR-501-5p, miR-101, miR-574-5p*	[72]

(continued)

Table 4.1 (continued)

	MicroRNAs		
Comparison	Up	Down	Ref
Double vs. single *KIT* mutant	*let-7d, miR-455-3p, miR-93, miR-106b, miR-130b, miR-103, miR-660, miR-99b, miR-107, miR-210, miR-720, miR-1260, miR-24, miR-151-3p, miR-1280, miR-342-3p, miR-125a-5p, miR-130a, miR-199a-5p, let-7e, miR-1274b, miR-362-5p, miR-25, miR-27b, miR-140-5p, let-7f, miR-1274a, miR-21, miR-886-3p, miR-146a, miR-30d, miR-331-3p, miR-143, miR-324-5p, miR-199a-3p, let-7a, miR-151-5p, miR-181a, miR-22, miR-30b, miR-140-3p, miR-23b, miR-17, miR-214, miR-181b, miR-361-5p, miR-132, let-7i*	*miR-940, miR-939, miR-150-3p, miR-638, miR-134, miR-1225-5p, miR-762, miR-572, miR-1275, miR-1207-5p, miR-1224-5p, miR-1268, miR-1915, miR-663, miR-1202, miR-296-5p, miR-1249, miR-1228, miR-188-5p, miR-1238, miR-101, miR-139-3p, miR-574-5p, miR-150-5p*	[72]

GIST gastrointestinal stromal tumor, *GI-LMS* gastrointestinal leiomyosarcomas, *e.g.* for example, *vs*. versus, *Ref* Reference
Note: Only top ranked differentially expressed miRNAs are listed. Up- and downregulated microRNAs refer to the first group in each comparison

in GIST [66, 67]. These microRNAs have been shown to target the two key factors *KIT* and *ETV1* in GIST tumorigenesis [67] (Table 4.2), suggesting that lower expression of these microRNAs in GIST could be important for the pathogenesis of this tumor type. The current known microRNAs involved in regulating key genes in GIST development and progression are shown in Fig. 4.2.

MicroRNAs Associated with Clinical and Pathological Features in GIST

Morphology, clinical behavior, and molecular biology of GISTs differ according to their anatomical localization [68]. Likewise, microRNA expression profiles of GISTs located in stomach are distinct from the GISTs found in small intestine [36, 37]. Notably, different sets of microRNAs associated with anatomical location were observed in different studies. For example, Haller et al. showed that gastric GISTs presented higher expressions of *miR-504*, *miR-7-1**, *miR-598*, and *miR-24-1**, while the intestinal GISTs had higher levels of *miR-220c*, *miR-229*, *miR-370*,

Table 4.2 Examples of aberrantly expressed microRNAs with functional role in GIST

microRNA	Target	Cellular function	Ref
miR-196a	*ANXA1*	Invasion	[69]
miR-494	*KIT*	Proliferation, apoptosis	[74]
miR-221/222	*KIT*	Proliferation, apoptosis	[67]
miR-17	*ETV1*	Proliferation, apoptosis	[67]
miR-20a	*ETV1*	Proliferation, apoptosis	[67]
miR-137	*TWIST1*	EMT, migration, cell cycle arrest, apoptosis	[73]
miR-125a-5p	*PTPN18*	Imatinib resistance	[72]
miR-218	*KIT*	Imatinib resistance, proliferation, invasion, apoptosis	[75, 101]

EMT Epithelial-mesenchymal transition, *Ref* Reference

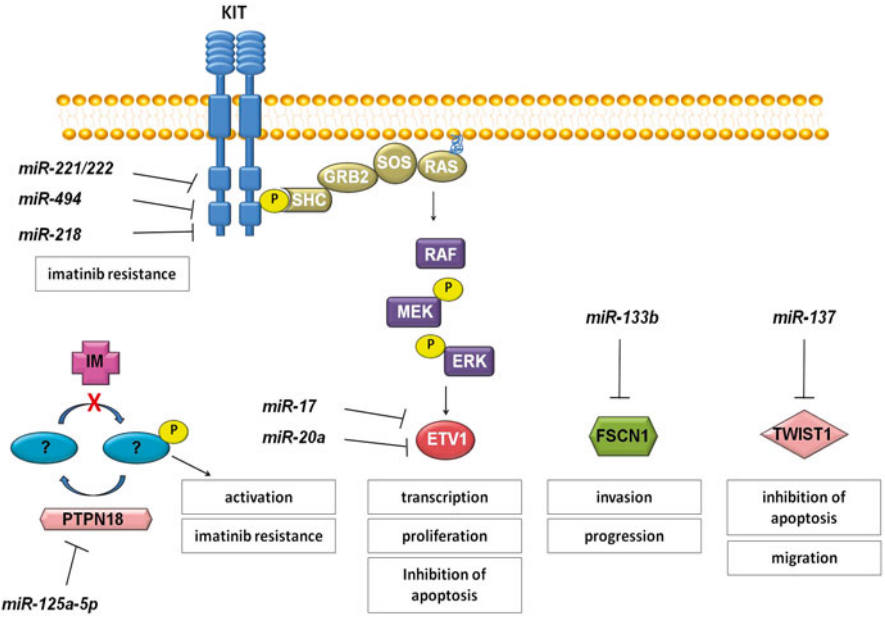

Fig. 4.2 MicroRNAs involved in the regulation of GIST development, progression, and imatinib response. In brief, *miR-221*, *miR-222*, *miR-494*, and *miR-218* directly target the *KIT* expression, while *miR-17* and *miR-20a* regulates the survival factor *ETV1*. *miR-133b* and *miR-137* regulate GIST progression by targeting *FSCN1* and *TWIST1*, respectively. *miR-125a-5p* regulates imatinib response through the regulation of *PTPN18*. *miR-218* also regulates imatinib response. *IM* Imatinib, *P* Phosphate group

miR-210, *miR-409-3p*, *miR-376a*, and *miR-376c* [37]. Choi et al. demonstrated higher expressions of *miR-383*, *miR-136*, *miR-146a*, and *miR-409a-3p*, and lower expressions of *miR-124a, miR-199b, miR-451, miR-663, miR-10a*, and *miR-218* in the intestinal compared to gastric GISTs [36]. The discrepancy is likely due to additional factors (e.g., risk grade and mutation status) that may contribute to differences besides anatomical locations in the tumors analyzed in both studies.

Several microRNA signatures have been described in GIST progression. In terms of tumor-risk group, a number of studies revealed distinct microRNA expression patterns between the high-risk and the low-risk GISTs, and identified a number of tumor-risk associated microRNAs (Table 4.1) [36, 69–71]. In the study of Choi et al., they compared microRNA profiles of 10 high-risk and 4 low-risk GISTs, and identified 28 microRNAs to be expressed at lower level in the high-risk group [36]. Yamamoto et al. reported 24 microRNAs with lower expression in the high-risk GISTs compared to low-to-intermediate risk tumors [70]. Kelly et al. found only *miR-150* to be expressed at higher level in the low-risk tumors [71], and Niinuma et al. reported higher *miR-196a* expression in the high-risk group [69].

Besides tumor risk, several microRNAs are associated with tumor metastasis in GISTs. For example, low expression of *miR-150-3p* and high expressions of *miR-301a-3p* and *miR-196a* are associated with metastasis in GIST [69, 72]. In experimental cell culture systems, two microRNAs have been evaluated for their effect on tumor progression. Overexpression of *miR-137* can inhibit cell migration and regulates epithelial-to-mesenchymal transition (EMT) by targeting *TWIST1* [73], and inhibition of *miR-196a* can suppress cell invasion in GIST cells [69].

In terms of survival, low expression of *miR-1915* is associated with disease-free and overall survival [72], while higher *miR-196a* expression is associated with poorer overall survival of GIST patients [69].

MicroRNAs Associated with Chromosomal and Genetic Alterations in GIST

As previously described, loss of 14q is common in GIST [31–33]. Downregulation of multiple microRNA clusters located at chromosome 14q (i.e., 14q32.31 and 14q32.33) has been reported in GISTs with 14q loss (Table 4.1) [36, 37]. One of the microRNAs located in this region, i.e., *miR-494*, was shown to directly target *KIT* and suppress its expression, and activates downstream signaling components such as AKT and STAT3 [74]. Functionally, inhibition of *miR-494* suppresses proliferation and induces apoptosis in GIST cells [74].

Given that *KIT* and *PDGFRA* are key factors involved in GIST tumorigenesis, microRNA-mediated regulation of these factors is important for GIST development. As aforementioned, *miR-494*, *miR-221*, and *miR-222* have been shown to directly regulate KIT expression in GIST cells [67, 74]. Recently, *miR-218* was also found directly targeting *KIT*, and its overexpression suppresses proliferation and invasion, and induces apoptosis in GIST-T1 cells [75]. On the other hand, *PDGFRA* is known to be regulated by several microRNAs in different cell types, such as *miR-126* in osteoblasts [76], *miR-34a* in gastric cancer [77], lung cancer [78], and glioma [79], and *miR-146a/146b-5p* in endothelial [80] and hematopoietic cells [81]; however, no microRNA has been experimentally validated to target *PDGFRA* in GIST.

GISTs show differential microRNA expression patterns according to their mutation status [37]. Several microRNAs are associated with *KIT* or *PDGFRA*-mutated

GISTs. For example, *miR-132*, *miR-766*, *miR-652*, *miR-629*, *miR-200c*, *miR-342-3p*, *miR-185*, *miR-146b-5p*, and *miR-150* levels are higher, whereas *miR-330-3p* is lower in *PDGFRA*-mutated GISTs as compared to *KIT*-mutated GISTs [37]. Higher expressions of *miR-221* and *miR-222* were found in the wild-type tumors compared to the tumors with *KIT* or *PDGFRA* mutation [37]. Concordantly, several studies have also revealed distinct mRNA expression profiles between GISTs with *KIT* and *PDGFRA* mutations [66, 82]. These findings suggest that, despite the common pathways activated by both mutations (e.g., PI3K/AKT and MAPK) [15], differences exist in the signal transduction networks between GISTs with *KIT* and *PDGFRA* mutations. In addition, several microRNAs are differentially expressed between GISTs with a single and double *KIT* mutations [72], suggesting that these microRNAs may be involved in partly distinct pathways [72].

Besides *KIT* and *PDGFRA*, several microRNAs are also associated with *SDHB* mutation. The *SDHB*-mutated GISTs show several microRNAs with higher (*miR-132*, *miR-146a*, *miR-193b*, *miR-193b**, *miR-455-3p*, *miR-455-5p*, *miR-484*, and *miR-886-5p*) and lower (*miR-125b*, *miR-450b*, *miR-488**, *miR-542-3p*, *miR-551b*, *miR-576-3p* and *miR-769-5p*) expressions compared to non-*SDHB*-mutated tumors [71].

MicroRNAs in Imatinib Resistance in GIST

MicroRNAs are known to play a role in tyrosine kinase inhibitor resistance [83–88]. The best example is the EGFR-inhibitor resistance in lung cancer. Numerous microRNAs (e.g., *miR-205*, *miR-374a*, *miR-548b*, *miR-30b*, *miR-30c*, *miR-221*, *miR-222*, and *miR-200* family members) have been shown to regulate EGFR-inhibitor response in lung cancer [84, 85, 89, 90]. In chronic myelogenous leukemia (CML), *miR-17–19b*, *miR-30e*, *miR-203*, and *miR-138* have been demonstrated to modulate imatinib sensitivity, while *miR-30a* promotes autophagy that enhances imatinib resistance [91–99].

In GIST, only two microRNAs have been functionally determined to modulate imatinib response [72, 100], despite a number of microRNAs are associated with imatinib resistance [72]. The expression of *miR-218* is lower in imatinib-resistant compared to -sensitive GIST cell lines. Overexpression of *miR-218* increases imatinib-induced cell death in the imatinib-resistant GIST430 cells. On the other hand, inhibition of *miR-218* expression increases cell viability and decreases apoptosis in the imatinib-sensitive GIST882 cells upon imatinib treatment. Although no target gene(s) of *miR-218* was identified, the authors propose that the effect might be mediated through PI3K/AKT signaling pathway.

The second microRNA is *miR-125a-5p*, which was found at higher expression levels in the imatinib-resistant than the -sensitive GISTs [72]. Overexpression of *miR-125a-5p* increases cell viability in the single *KIT*-mutated GIST882 cells upon imatinib treatment. However overexpression or suppression of *miR-125a-5p* in the double *KIT*-mutated GIST48 cells has no effect on imatinib response, suggesting

that microRNA-mediated regulation is an alternative resistance mechanism to secondary *KIT* mutations in GIST. Ectopic expression of *miR-125a-5p* suppresses its target gene *PTPN18* expression and silencing of PTPN18 increases cell viability in GIST882 cells upon imatinib treatment. The authors also observed an increased expression of *miR-125a-5p* and a decreased expression of PTPN18 in the imatinib-resistant subclone of GIST882 cells as compared to its sensitive counterpart, providing the functional evidence of *miR-125a-5p*-mediated regulation in imatinib resistance. PTPN18 is a member of the PEST domain containing protein-tyrosine phosphatase superfamily, which has been shown to dephosphorylate the phosphotyrosine residues of several tyrosine kinases, such as HER2 and SRC [101, 102]. Takahashi et al. recently demonstrated that altered phosphorylation of tyrosine kinases is an alternative mechanism of imatinib resistance in GIST [63]. Further studies have yet to determine whether the tyrosine kinases described by Takahashi et al. could be the substrate(s) of PTPN18.

Clinical Implications of microRNAs in GIST

MicroRNA expression profiles can distinguish GISTs from other sarcomas, and distinct microRNA expression signatures are associated with clinical, molecular, and histopathological features of GIST. These findings suggest a promising role for microRNAs as diagnostic and prognostic indicators in GIST.

Given their relatively higher stability in clinical samples and robust expression patterns, microRNAs have been suggested to have a greater utility as biomarkers in comparison to mRNAs [103]. Importantly, microRNAs can be released into the body fluids through microvesicles, which gives them a potential value as noninvasive biomarkers [104, 105]. Future studies evaluating the potential of circulating microRNAs as response markers for treatment or as reflective markers of GIST biological outcome would have a clinical benefit. However, there are some obstacles for circulating microRNAs, e.g., identification of an appropriate endogenous control and fluctuations in microRNA expression caused by diet, infection, treatment, trauma, or other factors [106].

Inhibition of KIT and PDGFRA by imatinib is the key therapeutic approach for advanced GISTs beside surgery. However, imatinib resistance is one of the biggest challenges in current GIST clinical practice. Post-transcriptional inhibition of oncogenes by microRNA mimics and activation of tumor suppressor genes by microRNA inhibitors are currently under investigation for their potential as therapeutic agents in cancer. KIT-targeting microRNA mimics (e.g., *miR-221, miR-222, miR-494*) [67, 74] may be used directly to target GIST cells to enhance the effect of imatinib for the purpose of overcoming resistance. Likewise, microRNA mimics/inhibitors for microRNAs specific to imatinib resistance, metastasis, risk grade or survival may be used for therapeutic purposes. Off-target effects and delivery of these molecules to specific GIST tissues/cells remain as the biggest challenges. Several strategies have

been developed for delivery of microRNA-based therapeutics, including the use of nanoparticles, liposomes, antibodies and nucleic acid structure modifications [107].

Conclusion

In the last 20 years, growing knowledge of GIST molecular biology has revolutionized the clinical management of this disease, from a treatment-resistant uncontrolled disease to the development of targeted therapies. Despite tyrosine kinase inhibitors improve the outcome of the majority of patients, they fail to provide a permanent cure and resistant clones are observed in most of the initially responding tumors.

Development of alternative treatment strategies is needed in order to overcome resistance to ATP-competitive kinase inhibitors. Complete understanding of molecular biology in GIST development, progression, and treatment response is necessary to establish a ground for developing effective combinational therapies with a goal of not only to temporarily control the disease, but also to permanently eradicate all tumor cells.

MicroRNAs have been shown to play a role not only in the GIST tumorigenesis, but also in the stratification of patients at risk of developing the disease or therapy response. Although this research area is still relatively understudied, the work reported in the last 3 years is indicative of the excitement in this area. Ongoing and future studies will illuminate effectiveness and safeness of microRNAs as novel agents for GIST treatment and their predictive value as novel biomarkers. This will hopefully turn GIST from a model of targeted therapies that control the disease progression to a model of complete cancer cure.

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