MicroRNAs: Promising chemoresistance biomarkers in gastric cancer with diagnostic and therapeutic potential

Christiane Matuszcak, Joerg Haier, Richard Hummel, Kirsten Lindner

Abstract
Gastric cancer (GC) is the fourth most common cancer worldwide and ranks second in global cancer mortality statistics. Perioperative chemotherapy plays an important role in the management and treatment of advanced stage disease. However, response to chemotherapy varies widely, with some patients presenting no or only minor response to treatment. Hence, chemotherapy resistance is a major clinical problem that impacts on outcome. Unfortunately, to date there are no reliable biomarkers available that predict response to chemotherapy before the start of the treatment, or that allow modification of chemotherapy resistance. MicroRNAs (miRNAs) could provide an answer to this problem. miRNAs are involved in the initiation and progression of a variety of cancer types, and there is evidence that miRNAs impact on resistance towards chemotherapeutic drugs as well. This current review aims to provide an overview about the potential clinical applicability of miRNAs as biomarkers for chemoresistance in GC. The authors focus in this context on the potential of miRNAs to predict sensitivity towards different chemotherapeutics, and on the potential of miRNAs to modulate sensitivity and resistance towards chemotherapy in GC.

Key words: MicroRNAs; Biomarker; Chemoresistance; Gastric cancer; Diagnostic; Therapeutic

Core tip: MicroRNAs (miRNAs) are a relatively new class of gene expression regulators, being involved in cancer initiation and progression. There is evidence that miRNAs impact on resistance towards various chemotherapeutics and are accessible and detectable in different tissue types, including blood samples, with great stability. Taken together, miRNAs seem to have great potential as new biomarkers for diagnostic and therapeutic approaches. This current review aims to provide an overview about the potential clinical applicability of miRNAs as biomarkers for chemoresistance in gastric cancer (GC), focusing on prediction and modulation of sensitivity and resistance towards chemotherapeutic drugs in GC.

Matuszcak C, Haier J, Hummel R, Lindner K. MicroRNAs: Promising chemoresistance biomarkers in gastric cancer with diagnostic and therapeutic potential. World J Gastroenterol 2014; 20(38): 13658-13666 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i38/13658.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i38.13658

INTRODUCTION
Gastric cancer (GC) is the fourth most common cancer worldwide and ranks second in global cancer mortality...
The prognosis of GC is poor with an overall 5-year survival rate of less than 35%[13], mostly caused by locally advanced disease at the date of initial diagnosis, reduced response to neoadjuvant or adjuvant therapy or tumor recurrence after surgical resection. Surgery is the preferred primary treatment option for the early stage of GC. Unfortunately, over 60% of the patients present advanced stage disease at the time point of diagnosis. In these cases, perioperative chemotherapy is widely applied based on the data of the MAGIC[40] and the ACCORD trials[8], including for example platinum- and fluoropyrimidine-based regimen or three-drug protocols with additional application of taxanes or anthracyclines[41,42]. These therapies are recommended nowadays even for patients with uT2 tumors[8,9]. However, response of the individual patient to this perioperative treatment varies widely, and some tumors are resistant to chemotherapeutic treatment completely. Hence, one main obstacle in the success of perioperative chemotherapy is the development of multi-drug resistance (MDR) in GC[10]. Based on the current literature, there are four major mechanisms that contribute to drug resistance in cancer cells: (1) decreased uptake of water soluble drugs[10]; (2) changes in intracellular pathways that affect the potential of cytotoxic drugs to kill cells, including alterations in the cell cycle, DNA repair, apoptosis pathways, metabolism/elimination of drugs, or others[10,12]; (3) increased energy-dependent efflux of hydrophobic drugs mediated via overexpression of a family of energy-dependent transporters (known as ATP-binding cassette transporters) such as P-glycoprotein 1 (P-gp, ABCB1) or breast cancer resistance protein (ABCG2) amongst others[10]; and (4) intracellular detoxifiers such as antioxidants (e.g., glutathione)[13,14]. Unfortunately, from a clinical point of view, there are so far no reliable biomarkers available that allow a prediction of response to chemotherapy in the individual patient before the start of treatment[15]. This leads to the problem that a number of patients undergo futile treatment with potential severe side-effects but without any benefit. If response to chemotherapy can be predicted before treatment, therapy could be better tailored via identification of patients that in fact profit from chemotherapy.

In this context, microRNAs (miRNAs) could provide a new approach for better clinical decision making. miRNAs are a relatively novel class of regulatory molecules that control translation and stability of messenger RNA (mRNAs) on a post-transcriptional level via interaction with the 3’-untranslated region (UTRs) of target mRNAs, that finally leads to destabilization and/or inhibition of their translation[16]. So far, over 2,500 human miRNAs have been identified according to the latest release (June 2013) of the miRBase database[17], and each single miRNA can potentially target up to hundreds of mRNAs[18]. miRNAs are involved in the regulation of almost all physiological processes such as cell development, differentiation, proliferation and apoptosis[19,20]. But more importantly, miRNAs have been found to impact on pathogenesis of a variety of human cancers. In this context, miRNAs can be roughly categorized into miRNAs that promote tumor development and growth (so-called oncomiRs), and miRNAs that inhibit tumor progression (so-called tumor-suppressor-miRs)[21,22]. Regarding GC, several (in vitro and in vivo) studies demonstrated according to the findings in other cancer types that tumors or GC cell lines present aberrant miRNA expression pattern compared to controls (normal gastric cell lines or samples from healthy patients)[23-31]. In conclusion, this data highly suggests that miRNAs may have an enormous potential as diagnostic[32], prognostic[33-35], or even therapeutic biomarkers[36,37].

This current review article now aims to summarize the evidence available so far about the impact of miRNAs on MDR in GC. We aim to specifically highlight from a clinical point of view, that miRNAs might help to predict tumor response to conventional chemotherapy, thereby providing a new approach as diagnostic biomarkers in GC. Moreover, we intend to show that miRNAs might further provide an enormous potential as therapeutic tools in the battle against GC by modulating and/or reversing MDR.

GENERAL CONSIDERATIONS ABOUT MIRNAs AS BIOMARKERS FOR CHEMoresISTANCE IN GC

**MiRNAs: Perfect candidates for biomarkers in clinical settings?**

The National Institutes of Health Biomarkers Definition Working Group defined a biomarker as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention”[38]. This definition of “a characteristic that is objectively measured and evaluated” implicates that molecular biomarkers which might be useful for prediction of the success of chemotherapy in cancer patients should (1) be easily detectable and assessable in clinical patient samples; and (2) be stably expressed and refractory to degradation in these samples in order to allow proper analysis on clinical samples. Only if these characteristics are present, miRNAs can further be assessed regarding their potential as biomarkers for response prediction.

And in fact, regarding the aspect of easily accessible clinical samples, there is a large body of evidence that shows that miRNAs are indeed detectable in a number of different sample types. For example, miRNAs can be found and analyzed in fresh frozen samples such as tumor biopsies or resection specimens, or in paraffin-embedded tissues[39]. Even more interesting from a clinical point of view, miRNAs can be detected as so-called “circularizing miRNAs” in a broad variety of human body fluids including urine[25], saliva[39], amniotic fluid[39] and pleural fluid[28] in healthy volunteers and cancer patients[35,40-43]. These circulating miRNAs are considered believed to originate either from immunocytes[44], as byproducts of
dead/dying cells[44-46], or to be secreted actively from tumor cells via microvesicles such as exosomes[47,48].

Regarding the stability of miRNA expression in clinical samples, Lu et al[49] reported that miRNAs are quite stable in different tissue samples (paraffin-embedded sections, rapid frozen samples) and present unique expression levels in different tissue types (salvia, blood, urine). Moreover, circulating miRNAs have been shown to be highly stable in the peripheral circulation caused for example by binding to RNA-binding proteins[48,49,51] or by their excretion in microvesicles[52,53]. In addition, Fang et al[54] reported that miRNAs present tissue-specific expression patterns with a reproducible and consistent expression. Finally, studies demonstrated that circulating miRNAs are fairly resistant to different pH solutions or repeated freeze-thawing cycles, and they are stable for 24 h at room temperature[55,56].

Taken together, miRNA expression patterns can successfully be assessed and analyzed in a variety of different samples, including biopsy or resection specimen, or blood samples, what supports the hypothesis that miRNAs could be used as clinically relevant diagnostic or therapeutic molecular biomarkers.

**MiRNAs: Do these molecules impact on MDR in general?**

Another aspect of using miRNAs as potential biomarkers for chemotherapy resistance in GC includes the possible impact on MDR in general. Only if there are data available which suggest that miRNAs modulate drug resistance in general, these molecules might be useful for evaluation of response prediction in GC patients.

And in fact, miRNAs have been proposed to play an important role in the development of MDR in cancer progression[55-58]. Despite the fact that this field of research is still in its infancy, it has been demonstrated that miRNAs are highly related to cancer progression (including growth arrest, invasion and metastasis) and are responsible for cancer-related inflammation, anticancer drug resistance and regulation of cancer stem cells[59]. Most interestingly, a number of miRNAs (such as members of the let-7 family, miR-16, miR-21 or miR-451 amongst others) have been confirmed to impact on more than one anticancer drug and/or to play a role in chemotherapy resistance in more than just one tumor type[57,60-62]. In addition, several anti-cancer-drugs target the same cancer-related genes that are targeted and regulated by miRNAs, implicating a link between chemotherapy induced cancer cell death and a modulation of chemotherapy resistance via miRNAs[63-65]. However, it has to be noted in this respect that the regulation of known cancer-related genes by miRNAs is much more frequently reported simply because these genes are well studied, and not all miRNAs show similar sensitivity/phenotypes in different cancer types. Finally, a number of transcripts from drug resistance-related genes may be targeted by more than one miRNA[66].

Taken together, the evidence available so far on miRNA analysis in different sample types, their stability in various tissues, and their involvement in chemotherapy resistance in general highly supports the hypothesis that miRNAs might be perfect candidates for biomarkers for chemotherapy resistance in GC in clinical settings.

**Limitations about the clinical use of miRNAs as biomarkers**

As miRNA research is so far mostly limited to in vitro experiments, its clinical applicability at this stage is somewhat limited, and there are a number of hurdles that need to be overcome before these molecules can be used in standard clinical settings.

With regards to sample acquisition and miRNA extraction for examples from blood samples, it has been demonstrated that several aspects and factors impact on results of miRNA analyses. These include the appropriate selection of samples (e.g., plasma or serum), collection tubes (EDTA, citrate, heparin), extraction methods (phenol/chloroform, silica: distinct differences in required fluid volume, yield, procedural contaminants etc), quality and quantity control, fasting or blood draw timing, all potentially. In addition, there are a number of different miRNA profiling methods including qRT-PCR, microarrays, sequence specific hybridization in solution followed by miRNA molecule counting based on reporter probes, and direct sequencing available, all of which with more or less relevant advantages and disadvantages regarding sensitivity and specificity, absolute quantification/accuracy and flexibility and throughput[67]. In general, it can be postulated, that there is a lack of standardized experimental techniques at this stage, and this impacts clearly on the possibility to compare data of different studies.

With regards to miRNAs as therapeutic tools, in vivo data on animal experiments are limited, and there are to our best knowledge so far only two clinical studies on a potential use of miRNAs as therapeutic tools in human beings available or on its way. Both studies do not refer to GC patients. In a phase 2a clinical trial, Janssen et al[68] evaluated the safety and efficacy of Miravirsen® (a locked nucleic acid-modified DNA phosphorothioate antisense oligonucleotide that sequesters mature miR-122 in a highly stable heteroduplex, thereby inhibiting its function) in 36 patients with chronic HCV genotype 1 infection. The authors could demonstrate prolonged dose-dependent reductions in HCV RNA levels without evidence of viral resistance and without dose-limiting adverse events and no escape mutations in the miR-122 binding sites of the HCV genome[69]. It is worth to mention that in two phase I safety studies in humans with Miravirsen® showed that Miravirsen® was well tolerated with no dose-limiting toxicities[70,71].

The second clinical trial (phase I trial), which is currently recruiting healthy patients as a control group, uses MRX34 (a liposome-formulated mimic of the tumor suppressor miR-34) in patients with inoperable primary liver cancer or metastatic cancer with liver involvement[72]. In this trial, the safety, pharmacokinetics and pharmacodynamics MRX34 is being evaluated in healthy patients[73].

Matuszcak C et al. MicroRNAs: Chemoresistance biomarkers in gastric cancer
Table 1 MicroRNAs as potential diagnostic biomarkers for chemoresistance in gastric cancer

| miRNAs      | Regulation | Drug resistance cell lines | Target          | Ref. |
|-------------|------------|----------------------------|-----------------|------|
| miR-15b     | Down ↓     | Vincristine                | PTEN            | [62] |
| miR-16      | Down ↓     | Vincristine                | PTEN            | [62] |
| miR-19a/b   | Up ↑       | Doxorubicin, vincristine   | PTEN            | [77] |
| miR-21      | Up ↑       | Cisplatin                  | PTEN            | [78] |
| miR-106a    | Up ↑       | Cisplatin                  | PTEN            | [79] |
| miR-181b    | Down ↓     | Cisplatin, vincristine     | BCL-2           | [82] |
| miR-497     | Down ↓     | Cisplatin, vincristine     | BCL-2           | [83] |
| miR-224     | Down ↓     | Cisplatin, vincristine     | BCL-2, XIAP     | [83] |
| miR-508-5p  | Down ↓     | Vincristine, doxorubicin   | ABCB1, ZNRD1    | [81] |

The table presents an overview about miRNAs that present different expression pattern between chemotherapy resistant gastric cancer cell lines and controls, including downstream targets and affected chemotherapeutic agents. PTEN: Phosphatase and Tensin homolog; BCL-2: B-cell lymphoma 2; XIAP: X-linked inhibitor of apoptosis protein; ABCB1: ATP-binding cassette sub-family B member 1; ZNRD1: DNA-directed RNA polymerase 1 subunit RPA12; miRNA: MicroRNA.

Taken together, there are many hurdles that need to be overcome before miRNAs can be safely applied as clinical biomarkers or therapeutic tools. Standardization of technical approaches, confirmation of in vitro results in animal experiments and finally safety assessment and treatment trials in humans have to show whether these molecules find their way into the daily clinic. However, first results are promising indeed.

**MiRNAs AND MDR IN GC**

There are numbers of potential applications for miRNAs as clinically relevant biomarkers. In the context of this review, we focus on two distinct aspects: miRNAs as diagnostic biomarkers, and miRNAs as therapeutic biomarkers.

MiRNAs as diagnostic biomarkers have to meet various requirements, some of which have been discussed in detail in the section above. However, the main requirement of a diagnostic biomarker in cancer is to distinguish between different “subgroups” of patients. For example, patients with cancer should reliably be distinguishable from patients with precancerous conditions or from non-cancer controls. In this context, a “simple comparison” of miRNA expression pattern between different “subgroups” of patients is needed in order to allocate patients into the respective “subgroups”, and at this stage, there is no need for further information about the exact impact of the respective miRNAs on tumor growth, metastasis, etc.

In contrast to this “simple analytic” approach, the use of miRNAs as therapeutic biomarkers mandates different key features: these miRNAs might not be differentially expressed between different subgroups of patients (such as responders and non-responders to chemotherapy). However, manipulation of the levels of these miRNAs should lead to changes in the cellular response of tumors to anticancer treatment, increasing thereby for example drug resistance. This means that for therapeutic biomarkers, there is a need for further knowledge about the exact mechanisms by which the respective miRNAs finally affect cancer cell behavior. The objective in the establishment of a therapeutic biomarker is to provide a more individualized therapy approach for every tumor type in the future.

**MiRNAs as promising biomarkers for chemoresistance in GC with diagnostic potential**

The potential to predict response to chemotherapy treatment is a promising clinical application of miRNAs. Successful prediction of treatment response would allow a more tailored, individual therapy planning, as patients who do not respond to this treatment modality would not have to undergo futile treatment with potential severe side-effects. However, response prediction of chemoresistance implies that miRNAs exhibit significant and reproducible differences in expression between patients that respond well to chemotherapy compared to patients that show no or minimal response to this treatment option. With other words, miRNA expression pattern should differ between chemotherapy resistant GC tumors and sensitive tumors.

And in fact, some first authors reported results from in vitro experiments that showed differing expression pattern of several miRNAs in chemotherapy resistant GC cell lines when compared to sensitive controls (Table 1), with some miRNAs being upregulated in resistant cancer cells, and others being downregulated. For example, Wang et al. demonstrated an upregulation of miR-19a/b in a vincristine resistant GC cell line (SCG7901/VCR) and a doxorubicin resistant GC cell line (SGC7901/ADR). In addition, Yang et al. reported that miR-21 was upregulated in cisplatin resistant GC cells (SCG7901/DDP) vs controls. Furthermore, miR-106a was shown to be upregulated in a cisplatin resistant GC cell line (SCG7901/DDP). Finally, miR-195 and miR-378 were found to be upregulated in 5-azacytidine resistant GC cell lines (MGC803, SGC7901 and AGS).

Xia et al. on the other hand showed that miR-15b and miR-16 both were downregulated in a vincristine resistant GC cell line (SGC7901), and Shang et al. showed that miR-508-5p was downregulated in two GC cell lines resistant towards doxorubicin (SGC7901/ADR) and vincristine (SGC7901/VCR). Furthermore, Zhu et al. demonstrated in vincristine resistant GC cell lines miR-181b, miR-200b/c and miR-497 to be downregulated compared to controls.

Finally, Wu et al. analyzed miRNA expression patterns in hydroxycamptothecin (HCPT)-resistant and HCPT-sensitive GC cell lines. miR-224 and miR-338-3p were only expressed in HCPT-resistant cells, and miR-141, miR-200a, miR-200b, miR-372, and miR-373 were only expressed in HCPT-sensitive cells.

Even more interesting from a clinical point of view, there is some first evidence that alterations in miRNA
expression pattern might be able to discriminate between responders and non-responders in clinical patient samples (in vivo). Kim et al\(^{85}\) for example presented data based on biopsy samples from 90 GC patients which were collected prior to chemotherapy. The authors identified a signature of several miRNAs (namely miR-363, miR-518f, miR-519e, miR-520a, and miR-520d) that was correlated to resistance to cisplatin and 5-fluorouracil therapy\(^{85}\). With regards to blood based circulating miRNAs, the current literature draws a promising picture: Zhu et al\(^{86}\) showed in a recent review including 22 studies with a sample size ranging from 37 to 164, that a total of 35 circulating miRNAs were differentially expressed between GC patients and healthy controls. Most interestingly, 6 of these miRNAs (miR-21\(^{78}\), -27a\(^{87}\), -106a\(^{89}\), -195\(^{86}\), -200c\(^{88}\) and -378\(^{88}\)) were shown to be deregulated in chemotherapy resistant GC cell lines as demonstrated in the in vitro experiments in the paragraph above, and to be potentially involved in MDR.

**miRNAs as promising biomarkers for chemoresistance in GC with therapeutic potential**

The application of miRNAs as modifiers of chemotherapy sensitivity is an even more interesting approach for a potential clinical use of these molecules, especially as additive treatment with conventional chemotherapeutics. This implicates that artificial manipulation of miRNA levels lead to increased sensitivity or reduced resistance towards various chemotherapeutic drugs. And again, there is growing evidence that modulation of miRNA expression in fact affects resistance of various tumors to chemotherapeutic treatment. However, as this field of research is still very young, results on GC are limited and refer so far only to in vitro experiments. In vivo studies on human patients with GC are not available yet.

Similarly to the data presented in the section about miRNAs with diagnostic potential, some miRNAs impact on sensitivity towards chemotherapy if their levels were artificially upregulated, others if their levels were downregulated. For example, upregulation of miR-21 or miR-106a was demonstrated to increase cisplatin resistance of GC cells\(^{78}\), and Deng et al\(^{86}\) showed that upregulation of miR-195 or miR-378 led to an enhanced 5-azacytidine resistance in normal gastric cells. Upregulation of miR-449 or miR-508-5p was demonstrated to positively impact on sensitivity towards cisplatin (miR-449) respectively vincristine or doxorubicin (miR-508-5p)\(^{81,88}\). Interestingly, in accordance with these findings about the modulation of sensitivity towards chemotherapeutic drugs via miRNAs, Bandres et al\(^{89}\) reported that upregulation of miR-451 led to an increased sensitivity of cancer cells towards radiotherapy by down-regulating the macrophage migration inhibitory factor (MIF).

Only one research group reported on the effect of miRNA downregulation on chemotherapy resistance: Zhao et al\(^{87}\) found that increased doxorubicin sensitivity in GC cells is connected with downregulation of miR-27a.

The authors of the aforementioned studies performed additional experiments in order to elucidate the underlying mechanisms by which the respective miRNAs finally impacted on sensitivity and resistance towards the different chemotherapeutic drugs. Table 2 and Figure 1 present an overview about the results of these investigations, and highlight the downstream targets and pathways that mediate the effects of manipulated miRNA expression in GC cells and their response to anticancer treatment.

**CONCLUSION**

miRNAs are an astonishing new class of regulators of global gene expression, and they affect tumor initiation and progression in a large number of malignancies. Moreover, these molecules were shown to impact on sensitivity towards various chemotherapeutic agents in a variety of cancers. Based on the fact that miRNAs play an important role in the development and regulation of MDR, and in addition are accessible and detectable in different tissue types including blood samples with great stability, these molecules seems to have great potential as new biomarkers for diagnostic and therapeutic approaches. In the context of this current review, we presented an overview about the data available so far on this new aspect of miRNAs as biomarkers for chemoresistance in GC. Despite the fact that data on this topic is still somewhat limited, the first reports on miRNAs as po-

---

**Table 2** MicroRNAs as potential therapeutic biomarkers for chemoresistance in gastric cancer

| miRNAs     | Regulation | Drug resistance | Target in GC | Drug treatment | Ref.  |
|------------|------------|-----------------|--------------|---------------|-------|
| miR-21     | Up↑        | Up↑             | PTEN         | Cisplatin     | [78]  |
| miR-27a    | Down↓      | Down↓           | Cyclin-D1, p21| Vincristine, 5-fluorouracil, cisplatin, doxorubicin | [87]  |
| miR-106a   | Up↑        | Up↑             | PTEN         | Cisplatin     | [79]  |
| miR-195    | Up↑        | Up↑             | CDK6, VEGF   | 5-azacytidine | [80]  |
| miR-378    | Up↑        | Up↑             | CDK6, VEGF   | 5-azacytidine | [80]  |
| miR-450    | Up↑        | Down↓           | BCL-2, CCDN1 | Cisplatin     | [88]  |
| miR-508-5p | Up↑        | Down↓           | ABCB1, ZNRD1 | Vincristine, doxorubicin | [81]  |

The table presents an overview about miRNAs that impact on chemotherapy resistance in gastric cancer cell lines, including downstream targets and affect-ed chemotherapeutic agents. PTEN: Phosphatase and Tensin homolog; CDK6: Cyclin-dependent kinase 6; VEGF: Vascular endothelial growth factor; BCL-2: B-cell lymphoma 2; CCDN1: Cyclin-D1 gene; ABCB1: ATP-binding cassette sub-family B member 1; ZNRD1: DNA-directed RNA polymerase I subunit. RPA12: MiRNA: MicroRNA.
Potential clinical biomarkers that predict sensitivity towards chemotherapeutic drug are very promising. We found a number of studies that demonstrated various miRNAs to be indeed associated with chemoresistance by presenting different expression patterns between resistant and sensitive tumors, both in vitro and in vivo. Furthermore, several research groups could provide first evidence that manipulation of the expression of certain miRNAs lead to changes in the sensitivity and resistance of GC towards chemotherapeutics in vitro. Taken together, this data draw a very promising picture of miRNAs as potential new biomarkers for chemoresistance in GC with diagnostic and therapeutic potential. This would help to tailor cancer therapy more individually, and to specifically select patients for chemotherapy that would profit from this treatment option. Further research on this highly interesting and clinically most relevant aspect of a potential use of miRNAs is highly warranted.

REFERENCES

1 Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011; 61: 69-90

2 Wilke MS. Therapie beim Magenkarzinom. Chirurg 2009; 80: 1023-1027

3 Robb WB, Mariette C. Predicting the response to chemotherapy in gastric adenocarcinoma: who benefits from neo-adjuvant chemotherapy? Recent Results Cancer Res 2012; 196: 241-268 [PMID: 23129379 DOI: 10.1007/978-3-642-31629-6_17]

4 Cunningham D, Allum WH, Stenning SP, Thompson JN, Van de Velde CJ, Nicolson M, Scarffe JH, Loffs FJ, Falk SJ, Iveson TJ, Smith DB, Langlely RE, Verma M, Weeden S, Chua YJ, Perioperative chemotherapy versus surgery alone for resectable gastroesophageal cancer. N Engl J Med 2006; 355: 11-20 [PMID: 16829592 DOI: 10.1056/NEJMoa055531]

5 Boige V, Pignon J, Saint-Aubert B, Lasser P, Conroy T, Bouché O, Segol P, Bedenne L, Rougier P, Ychou M. Final results of a randomized trial comparing preoperative 5-fluorouracil (F)/cisplatin (P) to surgery alone in adenocarcinoma of stomach and lower esophagus (ASLE): FNCLCC ACCORD07-FCCD 9703 trial. J Clin Oncol 2007; 25 Suppl: 4510

6 Ychou M, Boige V, Pignon JP, Conroy T, Bouché O, Lebreton G, Ducoutieux M, Bedenne L, Fabre JM, Saint-Aubert B, Genève J, Lasser P, Rougier P. Perioperative chemotherapy compared with surgery alone for resectable gastroesophageal adenocarcinoma: an FNCLCC and FFCD multicenter phase III trial. J Clin Oncol 2011; 29: 1715-1721 [PMID: 21444866 DOI: 10.1200/JCO.2010.33.0597]
Kasper S, Schuler M. Targeted therapies in gastroesophageal cancer. *Eur J Cancer* 2014; 50: 1247-1258 [PMID: 24495747 DOI: 10.1016/jejca.2014.01.009]

Oba K, Paolella S, Alberts S, Bang YJ, Benedetti J, Bleiberg H, Catimel D, Ferkic D, Michiels S, Morita S, Ohashi Y, Pignon JP, Rougier P, Sasaki M, Sakamoto J, Sargent D, Shiota K, Cutsem EV, Buyle M, Burzykowski T. Disease-free survival as a surrogate for overall survival in adjuvant trials of gastric cancer: a meta-analysis. *J Natl Cancer Inst* 2013; 105: 1600-1607 [PMID: 24108812 DOI: 10.1093/jnci/djt270]

Ronnellenfisch U, Schwarzbach M, Hoftehne R, Kienle P, Kiersch M, Slanger TE, Jensen K. Perioperative chemo(radio)therapy versus primary surgery for resectable adenocarcinoma of the stomach, gastroesophageal junction, and lower esophagus. *Ehrhage Database Spezial* 2013; 5: CD008107 [PMID: 23728671 DOI: 10.1002/14651858.CD008107.pub2]

Szakács G, Jakab K, Antal F, Sarkadi B. Diagnostics of multidrug resistance in cancer. *Pathol Oncol Res* 1998; 4: 251-257 [PMID: 9897354 DOI: 10.1007/BF02905214]

Rabik CA, Dolan ME. Molecular mechanisms of resistance and toxicity associated with platinating agents. *Cancer Treat Rev* 2007; 33: 9-23 [PMID: 17084534 DOI: 10.1016/j.ctrv.2006.09.006]

Johnstone RW, Ruelli AA, Tainton KM, Smyth MJ. A role for P-glycoprotein in regulating cell death. *Leuk Lymphoma* 2000; 38: 1-11 [PMID: 10811443]

Harle BD. MicroRNAs in vertebrate development. *Curr Opin Genet Dev* 2005; 15: 410-415 [PMID: 15979305 DOI: 10.1016/j.gde.2005.06.012]

Hwang H, Hwang Y, Lee S, Lee D. Rule-based multi-scale simulation for drug effect pathway analysis. *BMC Med Inform Decis Mak* 2013; 13 Suppl 1: S4 [PMID: 23566173 DOI: 10.1186/1472-6947-13-S1-S4]

Pietrantonio F, De Braud F, Da Prat V, Perrone F, Pierotti MA, Gariboldi M, Fanetti G, Biondani P, Pellegrinelli A, Bossi I, Di Bartolomeo M. A review on biomarkers for prediction of treatment outcome in gastric cancer. *Anticancer Res* 2013; 33: 1257-1266 [PMID: 2394763]

David S, Meltzer SJ. MicroRNA involvement in esophageal carcinogenesis. *Curr Opin Pharmacol* 2011; 11: 612-616 [PMID: 21992930 DOI: 10.1016/j.coph.2011.09.006]

Kozomara A, Griffiths-Jones S. miRBase: integrating microRNA annotation and deep-sequencing data. *Nucleic Acids Res* 2011; 39: D152-D157 [PMID: 21037258]

Li L, Yang F, Wang X, Hu J, Yang L, Tang C, Wu Y, Miao K, Liu R, Shu J. Effect of 15-hydroxyprostaglandin dehydrogenase gene on the proliferation of gastric cancer cell murine forestomach carcinoma. *Exp Ther Med* 2014; 7: 290-294 [PMID: 23434808 DOI: 10.3892/etm.2013.1404]

Barbarotto E, Schmittgen TD, Calin GA. MicroRNAs and cancer: profile, profile, profile. *Int J Cancer* 2008; 122: 969-977 [PMID: 18091838 DOI: 10.1002/ijc.23343]

Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell* 2005; 120: 15-20 [PMID: 15652477 DOI: 10.1016/j.cell.2004.12.035]

Esquela-Kerscher A, Slack FJ. Oncomirs - microRNAs with a role in cancer. *Nat Rev Cancer* 2006; 6: 259-269 [PMID: 16557279 DOI: 10.1038/nrc1840]

Calin GA, Croce CM. MicroRNA signatures in human cancers. *Nat Rev Cancer* 2006; 6: 857-866 [PMID: 17060945 DOI: 10.1038/nrc1870]

Allegro A, Alonci A, Campo S, Penna G, Petruangaro A, Gerace D, Musolin C. Circulating microRNAs: new biomarkers in diagnosis, prognosis and treatment of cancer (review). *Int J Oncol* 2012; 41: 1897-1912 [PMID: 23026890 DOI: 10.3892/ijo.2012.1647]

Guo LH, Li H, Wang F, Yu J, He JS. The Tumor Suppressor Roles of miR-433 and miR-127 in Gastric Cancer. *Int J Mol Sci* 2013; 14: 14171-14184 [PMID: 23880861 DOI: 10.3390/
MicroRNAs: Key Regulators of Oncogenesis. Babashah S. 

MicroRNAs as circulating biomarkers. J Cell Mol Med 2010; 14(6): 2317-2328. [PMID: 20439679 DOI: 10.1111/j.1749-6632.2010.08901.x]

MicroRNA-224 functions as an onco-miRNA in hepatocellular carcinoma cells by activating AKT signaling. Oncol Lett 2012; 4: 483-488. [PMID: 23741247 DOI: 10.3892/ol.2012.742]

MicroRNA Biomarkers in Cancer. J Lipid Res 2013; 54: 1174-1181. [PMID: 23505318 DOI: 10.1194/jlr.R034991]

MicroRNAs: Functional Aspects and Diagnostic Utility in Oncology. Int J Mol Sci 2013; 14: 4934-4968. [PMID: 23454666 DOI: 10.3390/ijms14094934]

MicroRNA expression profiles classify human cancers. Nature 2005; 435: 834-838. [PMID: 15944708 DOI: 10.1038/nature03702]

MicroRNA expression profiles in human cells and blood plasma. RNA Biol 2012; 9: 1066-1075. [PMID: 22858679 DOI: 10.4161/ma.21083]

MicroRNA expression profiles in human cells and plasma. J Pathol 2012; 229: 1174-1181. [PMID: 22819102 DOI: 10.1002/path.2836]

MicroRNAs in pancreatic cancer. J Clin Invest 2010; 120: 173-182. [PMID: 20361112 DOI: 10.1172/JCI43276]

MicroRNAs as circulating biomarkers in cancer. J Clin Cancer 2010; 19: 62-72. [PMID: 20377927 DOI: 10.1177/1533286009357154]

MicroRNAs as plasma biomarkers in pancreatic cancer. J Clin Oncol 2010; 28: 2156-2163. [PMID: 20398612 DOI: 10.1200/JCO.2009.25.8996]

MicroRNAs as potential biomarkers for early detection of liver cancer. J Gastrointest Oncol 2013; 4: 202-210. [PMID: 23703906 DOI: 10.2167/jgo120026]

MicroRNAs in gastric cancer. J Gastrointest Oncol 2012; 3: 119-126. [PMID: 22448413 DOI: 10.11187/jgo120026]

MicroRNAs in hepatocellular cancer. J Gastrointest Oncol 2012; 3: 119-126. [PMID: 22448413 DOI: 10.11187/jgo120026]

MicroRNAs in lung cancer. J Gastrointest Oncol 2012; 3: 119-126. [PMID: 22448413 DOI: 10.11187/jgo120026]

MicroRNAs in ovarian cancer. J Gastrointest Oncol 2012; 3: 119-126. [PMID: 22448413 DOI: 10.11187/jgo120026]

MicroRNAs in prostate cancer. J Gastrointest Oncol 2012; 3: 119-126. [PMID: 22448413 DOI: 10.11187/jgo120026]

MicroRNAs in breast cancer. J Gastrointest Oncol 2012; 3: 119-126. [PMID: 22448413 DOI: 10.11187/jgo120026]

MicroRNAs in colorectal cancer. J Gastrointest Oncol 2012; 3: 119-126. [PMID: 22448413 DOI: 10.11187/jgo120026]

MicroRNAs as circulating biomarkers in the detection of colorectal cancer. J Gastrointest Oncol 2012; 3: 119-126. [PMID: 22448413 DOI: 10.11187/jgo120026]

MicroRNAs as circulating biomarkers in the early detection of colorectal cancer. J Gastrointest Oncol 2012; 3: 119-126. [PMID: 22448413 DOI: 10.11187/jgo120026]

MicroRNAs as circulating biomarkers in the detection of colorectal cancer. J Gastrointest Oncol 2012; 3: 119-126. [PMID: 22448413 DOI: 10.11187/jgo120026]

MicroRNAs as circulating biomarkers in the detection of colorectal cancer. J Gastrointest Oncol 2012; 3: 119-126. [PMID: 22448413 DOI: 10.11187/jgo120026]
get biomarker diagnostics to market. *Biotechnol Healthc* 2010; 7: 22-25 [PMID: 22478826]

75 **Bhatt AN**, Mathur R, Farooque A, Verma A, Dwarakanath BS. Cancer biomarkers - current perspectives. *Indian J Med Res* 2010; 132: 129-149 [PMID: 20716813]

76 **Mäbert K**, Cojoc M, Peitzsch C, Kurth I, Souchelnytskyi S, Dubrovskova A. Cancer biomarker discovery: current status and future perspectives. *Int J Radiat Biol* 2014; 90: 659-677 [PMID: 24524284 DOI: 10.1016/j.ijrrb.2013.04.010]

77 **Wang F**, Li T, Zhang B, Li H, Wu Q, Yang L, Nie Y, Wu K, Shi Y, Fan D. MicroRNA-19a/b regulates multidrug resistance in human gastric cancer cells by targeting PTEN. *Biochem Biophys Res Commun* 2013; 434: 688-694 [PMID: 23603526 DOI: 10.1016/j.bbrc.2013.04.010]

78 **Yang SM**, Huang C, Li XF, Yu MZ, He Y, Li J. miR-21 confers cisplatin resistance in gastric cancer cells by regulating PTEN/Akt pathway. *Toxicology* 2013; 306: 162-168 [PMID: 23466500 DOI: 10.1016/j.tox.2013.02.014]

79 **Fang Y**, Shen H, Li H, Cao Y, Qin R, Long L, Xie C, Xu W. miR-106a confers cisplatin resistance by regulating PTEN/PI3K/AKT pathway in gastric cancer cells. *Acta Biochim Biophys Sin (Shanghai)* 2015; 48: 963-972 [PMID: 24108762 DOI: 10.1093/abbs/gmt106]

80 **Deng H**, Guo Y, Song H, Xiao B, Sun W, Liu Z, Yu X, Xia T, Cui L, Guo J. MicroRNA-195 and microRNA-378 mediate tumor growth suppression by epigenetical regulation in gastric cancer. *Gene* 2013; 518: 351-359 [PMID: 23333942 DOI: 10.1016/j.gene.2012.12.103]

81 **Zhu W**, Shan X, Wang T, Shu Y, Liu P. miR-181b modulates multidrug resistance by targeting BCL2 in human cancer cell lines. *Int J Cancer* 2010; 127: 2520-2529 [PMID: 20162574 DOI: 10.1002/ijc.25260]

82 **Zhu W**, Zhu D, Lu S, Wang T, Wang J, Jiang B, Shu Y, Liu P. miR-497 modulates multidrug resistance of human cancer cell lines by targeting BCL2. *Med Oncol* 2012; 29: 384-391 [PMID: 21258880 DOI: 10.1007/s12032-010-9797-4]

83 **Wu XN**, Shao XQ, Meng XX, Zhang XN, Zhu L, Liu SX, Lin J, Xiao HS. Genome-wide analysis of microRNA and mRNA expression signatures in hydroxyxycamptothecin-resistant gastric cancer cells. *Acta Pharmacol Sin* 2011; 32: 259-269 [PMID: 21293479 DOI: 10.1038/aps.2010.204]

84 **Kim CH**, Kim HK, Rettig RL, Kim J, Lee ET, Aprilekova O, Choi IJ, Munroe DJ, Green JE. miRNA signature associated with outcome of gastric cancer patients following chemotherapy. *BMC Med Genomics* 2011; 4: 79 [PMID: 22112324 DOI: 10.1186/1755-8794-4-79]

85 **Zhu W**, Zhu D, Lu S, Wang T, Wang J, Jiang B, Shu Y, Liu P. miR-497 modulates multidrug resistance of human cancer cell lines by targeting BCL2. *Med Oncol* 2012; 29: 384-391 [PMID: 21258880 DOI: 10.1007/s12032-010-9797-4]

86 **Yang SM**, Huang C, Li XF, Yu MZ, He Y, Li J. miR-21 confers cisplatin resistance in gastric cancer cells by regulating PTEN/Akt pathway. *Toxicology* 2013; 306: 162-168 [PMID: 23466500 DOI: 10.1016/j.tox.2013.02.014]

87 **Fang Y**, Shen H, Li H, Cao Y, Qin R, Long L, Xie C, Xu W. miR-106a confers cisplatin resistance by regulating PTEN/PI3K/AKT pathway in gastric cancer cells. *Acta Biochim Biophys Sin (Shanghai)* 2015; 48: 963-972 [PMID: 24108762 DOI: 10.1093/abbs/gmt106]

88 **Deng H**, Guo Y, Song H, Xiao B, Sun W, Liu Z, Yu X, Xia T, Cui L, Guo J. MicroRNA-195 and microRNA-378 mediate tumor growth suppression by epigenetical regulation in gastric cancer. *Gene* 2013; 518: 351-359 [PMID: 23333942 DOI: 10.1016/j.gene.2012.12.103]

89 **Shang Y**, Zhang Z, Liu Z, Peng B, Ren G, Li K, Zhou L, Sun Y, Li M, Zhou J, An Y, Wu K, Nie Y, Fan D. miR-508-5p regulates multidrug resistance of gastric cancer by targeting ABCB1 and ZNRD1. *Oncogene* 2014; 33: 3267-3276 [PMID: 23893241 DOI: 10.1038/onc.2013.297]

90 **Zhu W**, Shan X, Wang T, Shu Y, Liu P. miR-181b modulates multidrug resistance by targeting BCL2 in human cancer cell lines. *Int J Cancer* 2010; 127: 2520-2529 [PMID: 20162574 DOI: 10.1002/ijc.25260]

91 **Bandres E**, Bitarte N, Arias F, Agorreta J, Fortes P, Agirre X, Zarate R, Diaz-Gonzalez JA, Ramirez N, Solá JJ, Jimenez P, Rodriguez J, Garcia-Foncillas J. microRNA-451 regulates macrophage migration inhibitory factor production and proliferation of gastrointestinal cancer cells. *Clin Cancer Res* 2009; 15: 2281-2290 [PMID: 19318487 DOI: 10.1158/1078-0432.CCR-08-1818]

92 **Bandres E**, Bitarte N, Arias F, Agorreta J, Fortes P, Agirre X, Zarate R, Diaz-Gonzalez JA, Ramirez N, Solá JJ, Jimenez P, Rodriguez J, Garcia-Foncillas J. microRNA-451 regulates macrophage migration inhibitory factor production and proliferation of gastrointestinal cancer cells. *Clin Cancer Res* 2009; 15: 2281-2290 [PMID: 19318487 DOI: 10.1158/1078-0432.CCR-08-1818]

93 **Bandres E**, Bitarte N, Arias F, Agorreta J, Fortes P, Agirre X, Zarate R, Diaz-Gonzalez JA, Ramirez N, Solá JJ, Jimenez P, Rodriguez J, Garcia-Foncillas J. microRNA-451 regulates macrophage migration inhibitory factor production and proliferation of gastrointestinal cancer cells. *Clin Cancer Res* 2009; 15: 2281-2290 [PMID: 19318487 DOI: 10.1158/1078-0432.CCR-08-1818]

94 **Bandres E**, Bitarte N, Arias F, Agorreta J, Fortes P, Agirre X, Zarate R, Diaz-Gonzalez JA, Ramirez N, Solá JJ, Jimenez P, Rodriguez J, Garcia-Foncillas J. microRNA-451 regulates macrophage migration inhibitory factor production and proliferation of gastrointestinal cancer cells. *Clin Cancer Res* 2009; 15: 2281-2290 [PMID: 19318487 DOI: 10.1158/1078-0432.CCR-08-1818]

95 **Bandres E**, Bitarte N, Arias F, Agorreta J, Fortes P, Agirre X, Zarate R, Diaz-Gonzalez JA, Ramirez N, Solá JJ, Jimenez P, Rodriguez J, Garcia-Foncillas J. microRNA-451 regulates macrophage migration inhibitory factor production and proliferation of gastrointestinal cancer cells. *Clin Cancer Res* 2009; 15: 2281-2290 [PMID: 19318487 DOI: 10.1158/1078-0432.CCR-08-1818]
