MicroRNA profiling in cancer

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ABSTRACT

The diagnosis of cancer has undergone major changes in the last 40 years. Once based purely on morphology, diagnosis has come to incorporate immunological, cytogenetic and molecular methods. Many cancers, especially leukaemias, are now defined by molecular markers. Gene expression profiling based on mRNA has led to further refinement of the classification and diagnosis of cancer. More recently, miRNAs (microRNAs), among other small non-coding RNA molecules, have been discovered and found to be major players in cell biology. miRNAs, having both oncogenic and tumour-suppressive functions, are dysregulated in many types of cancer. miRNAs also interfere with metastasis, apoptosis and invasiveness of cancer cells. In the present review, we discuss recent advances in miRNA profiling in human cancer. We discuss both frequent and rare tumour types and give an outlook on future developments.

INTRODUCTION

Over the last four decades, major progress has been made in the treatment of cancer. Certain types of leukaemias and lymphomas and some solid tumours, such as testicular cancer, are now curable in the majority of cases or obtain long-lasting remissions. For other tumours, such as lung cancer and pancreatic cancer, limited progress has been made. Concomitant with the overall improved prognosis of cancer, the diagnostic methods have become more refined and sophisticated. Building on purely morphological diagnosis, the pathologists have added immunological, cytogenetic and molecular methods to their armamentarium. Certain types or subtypes of malignancies are now defined by molecular or cytogenetic aberrations. Examples are chronic myelogenous leukaemia [translocation {9;22}, which results in the expression of the bcr-abl (breakpoint cluster region/c-abl oncogene 1, non-receptor tyrosine kinase) oncogene], acute promyelocytic leukaemia [translocation {15;17} and PML-RARA (promyelocytic leukaemia-retinoic acid receptor α) rearrangement] and Burkitt’s lymphoma (c-myc translocation). At the same time, based on typical molecular or immunological markers, new and highly effective treatments have been developed for these malignancies.

Key words: cancer, gene expression profile, leukaemia, malignancy, microRNA, non-coding RNA.

Abbreviations: ABC, activated B-cell-type; AGO2, Argonaute2; ALL, acute lymphoblastic leukaemia; AML, acute myelogenous leukaemia; bcr-abl, breakpoint cluster region/c-abl oncogene 1, non-receptor tyrosine kinase; BRAF, v-raf murine sarcoma viral oncogene homologue B1; CDK, cyclin-dependent kinase; CLL, chronic lymphocytic leukaemia; CML, chronic myelogenous leukaemia; ERK, extracellular-signal-regulated kinase; GCB, germinal centre B-cell-like type; GEP, gene expression profile; HCC, hepatocellular carcinoma; IL-6, interleukin-6; JAK, Janus kinase; MAPK, mitogen-activated protein kinase; miRNA, microRNA; MLL, myeloid/lymphoid or mixed-lineage leukaemia; MMP, matrix metalloproteinase; mTOR, mammalian target of rapamycin; NF, nuclear factor; NHL, non-Hodgkin’s lymphoma; NK cell, natural killer cell; PED, phosphoprotein; PI3K, phosphoinositide 3-kinase; PTEN, phosphatase and tensin homologue deleted on chromosome 10; RISC, RNA-induced silencing complex; RT-qPCR, reverse transcription real-time quantitative PCR; siRNA, small interfering RNA; SNP, single nucleotide polymorphism; STAT, signal transducer and activator of transcription; TGF-β, transforming growth factor-β; TRAIL, tumour-necrosis-factor-related apoptosis-inducing ligand; USF2, upstream stimulatory factor 2; UTR, untranslated region.

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Cancer is often heterogeneous in both its presentation and its clinical course. In the late 1990s, based on RNA extracted from fresh tumour tissue, GEPs (gene expression profiles) were introduced to classify or segregate tumours with similar morphology or phenotypes. Since several thousand genes are involved, these profiles have helped in recognition of pathways involved in oncogenic transformation, but only in a few selected cases have they been helpful in clinical decision making. More recently, the discovery of miRNAs (microRNAs) has made a major impact on cell biology.

miRNAs are small RNA molecules (19–22 or 19–25 nucleotides) that originally were considered redundant. They control gene expression by destabilizing transcription. miRNAs also regulate gene expression by inhibiting the degradation of their target mRNAs and by inhibiting translation; this function occurs through pairing of miRNA base sequences (generally at the 5′ end) to partially or fully complementary sites on the mRNA (both in coding and non-coding regions, often within the 3′ end). Through regulation of gene expression, miRNAs are involved in multiple biological processes, including differentiation, proliferation, apoptosis and stress response. Up to 30% of human genes are regulated by miRNAs. miRNAs are largely conserved between species and in tissue or blood specimens; they are more resistant to degradation than mRNAs. At present, more than 1000 human miRNAs are known (see miRBase at http://www.mirbase.org, release 16, October 2010).

Most types of human cancer have dysregulated miRNAs. Calin et al. [1] mapped 186 miRNAs and compared their locations with the locations of previous reported non-random genetic alterations. They showed that miRNA genes are frequently located at fragile sites, in minimal regions of loss of heterozygosity, in minimal regions of amplification (minimal amplicons) or in common breakpoint regions. Overall, more than half of the miRNA genes studied were found to be in cancer-associated genomic regions or in fragile sites [1]. In this review, we will discuss miRNA profiling for human cancer, in general, and for different types of cancer. We will compare miRNA profiles with established GEPs and focus on recent data. Because of the large amount of literature on this topic, we cannot review every type of cancer or every publication.

**BIOGENESIS OF miRNAs**

The first step of miRNA biogenesis is the synthesis of pri-miRNA transcripts. The transcripts (which originate either from independent genes or as introns of protein-coding genes) are transcribed by RNA polymerase II. Subsequently, these pri-miRNAs are processed and cleaved within the nucleus by Drosha, an RNase III-type nuclease, creating hairpin structures of 60–70 nucleotides (pre-miRNAs). The enzymes processing miRNA precursors operate in complexes with double-stranded RNA-binding proteins. Some pre-miRNAs are produced from very short introns as a result of splicing. Ultimately, the pre-miRNAs are exported into the cytoplasm by the nuclear receptor exportin-5. The next step is further cleavage in the cytoplasm by Dicer and TRBP [TAR (transactivation-responsive RNA of HIV-1) RNA-binding protein]. Subsequently, an asymmetric duplex intermediate (miRNA:miRNA*) is formed. This duplex is then loaded into the RISC (RNA-induced silencing complex). One of the strands becomes active; the other strand, the passenger strand (miRNA*), is usually released and degraded. The active miRNA, within the RISC, is now ready to perform functions such as translational repression or mRNA degradation. Argonaute proteins directly interact with miRNAs, and GW182 proteins, which act as key factors in translational repression, are important factors in the assembly and function of RISCs. Base pairing of the miRNA with an mRNA is generally imperfect and targets sequences in the 3′-UTR (untranslated region) of the mRNA. Efficient targeting requires continuous base pairing of miRNA nucleotides 2–8 (the seed region) [2]. Interestingly, sometimes both the -3p and -5p forms of miRNAs (derived from the same precursor) are active and abundant in specific tissues. A recent example is miR-199a of which both the -3p and -5p forms were found to target the brm subunit of the SWI/SNF complex [3].

**METHODS USED FOR GENETIC PROFILING AND miRNAs**

Genetic profiling is performed on RNA from tumours using DNA chip technology. These chips contain arrays of oligonucleotides (bound to glass slides). Under high-stringency conditions, the oligonucleotides are hybridized to the target cDNA or cRNA. The relative abundance of the target sequences can be measured because they are labelled with a fluorochrome. Genetic profiling characterizes tumours based on their oligonucleotide ‘signatures’ and serves to classify the tumours based on pathways involved, treatment response, survival and other biological characteristics. The reproducibility and statistical methods involved in generating GEPs were critically discussed in a recent publication [4].

Similar to their use for GEPs, DNA chips are also commonly used for the analysis of miRNAs. Liu et al. [5] have described in detail the methods, equipment and reagents used for miRNA profiling using DNA arrays. There are several commercial platforms available for microarray-based miRNA profiling. Overall, these platforms are well comparable and reproducible. An
earlier study compared five different platforms testing 309 miRNA probes [6]. Although the authors found a high interplatform repeatability and comparability, some problems with concordance were described because of different stringencies in detection call and with normalization. A different and more recent study compared four platforms and found highly reproducible data [7]. Results from chip analysis have to be confirmed by RT-qPCR (reverse transcription quantitative real-time PCR). In many instances, when important miRNAs are already known, only RT-qPCR is used for profiling. Alternatives to the oligonucleotide arrays are bead-based fluorescent hybridization systems. Less commonly used is Northern blotting (which is time-consuming and less sensitive than RT-qPCR). In situ hybridization can detect the tissue localization of miRNAs. For an extensive review of methods used for miRNA profiling, see Ferdin et al. [8]. Earlier, a detailed description of all laboratory procedures for investigating miRNAs was published by Calin et al. [9]. Sequencing of the whole genome for the discovery of miRNAs is, at present, a research tool only. However, it is already clear that next generation sequencing technologies can detect, enrich and analyse miRNAs accurately [10].

miRNA PROFILING IN HAEMATOLOGICAL MALIGNANCIES

**CLL (chronic lymphocytic leukaemia)**

CLL has become the paradigmatic disease in which miRNA profiling has resulted in major new insights. CLL has a variable course. Some patients require little or no treatment, whereas in other patients, the disease is progressive and leads to the death of the patient despite multiple lines of treatment. Interphase cytogenticities and the mutational status of tumour cells provide some prognostic information. Classical GEP generally did not show dramatic differences between the different presentations or types of CLL but has resulted in the discovery of new markers such as ZAP-70, LPL and CLLU1 [11].

The homozygous or heterozygous deletion of the chromosomal region 13q14.3 occurs in more than half of cases of CLL and is associated with an indolent clinical course. For many years, it was suspected that a tumour suppressor gene was located in this region, but more recently it was shown that two genes encoding miRNAs (miR-15a and -16-1) are located in the region (a 30-kb region of loss). This finding suggests that miRNAs could be involved in the pathogenesis of human cancer. Indeed, in a collection of 120 CLL samples, the expression of miR-15a and -16 was decreased or absent [12]. These miRNAs map to a region between exons 2 and 5 of the *Leu2* gene. It was speculated that the loss of both miRNAs is an early or initiating event in the pathogenesis of CLL.

In continuation of this work to elucidate the pathogenesis of CLL, a unique miRNA signature for CLL was established [13]. A combination of nine miRNAs (expression profile miR-181 high, miR-155 high, miR-146 high, miR-24-2 high, miR-23b high, miR-23a high, miR-222 high, miR-221 high and miR-29c low) correlated with a short interval to progression. This profile is also associated with known biological risk factors for CLL, such as high ZAP-70 expression and unmutated IgVH (Ig variable region) genes. In the same study, germline or somatic mutations of some miRNA genes were found in 11 of 75 patients with CLL. This finding points to a genetic disposition for cancer in some patients with CLL. A mouse model of CLL also supports the role of certain miRNAs in the pathogenesis of CLL. In this model, a 3′ point mutation adjacent to miR-16-1 was discovered; it results in the reduced expression of miR-16-1. Taken together, miRNAs and other non-coding RNAs play an important role in CLL. The interplay between protein-coding genes, miRNAs and other non-coding RNAs in CLL was recently reviewed [14].

Visone et al. [15] examined the correlation between specific karyotypes and miRNA signatures. A total of 32 miRNAs were able to discriminate between five categories of CLL (deletion 11q, deletion 17p, trisomy 12, deletion 13q and normal karyotype). For nine miRNAs, the biological impact was studied by correlating miRNA expression with mRNA expression. In cases with 17p deletion, the genotype of unmutated immunoglobulin heavy chains, high ZAP-70 expression and low expression of four miRNAs (miR-223, -29c, 29b and miR-181 family) predicted progressive disease. Rossi et al. [16] developed a score incorporating miR-21 for stratifying patients with CLL and chromosome 17p deletions (21-F–K-score). Patients with a low score lived longer. In this study, when patients with 17p deletions and those with normal karyotypes were compared, miR-21, -34a, -155 and -181b were differentially expressed. miR-181b was especially down-regulated in treatment-refractory cases.

**Hodgkin’s lymphoma**

Hodgkin’s lymphoma is a paradigm for a curable cancer. This is especially true in early stages and for younger patients. Nevertheless, some patients relapse, and some ultimately die of refractory disease. The malignant cells (Hodgkin–Reed–Sternberg cells) are a minority in the involved lymph node and usually are surrounded by reactive cells. Most of the malignant cells are derived from B-cells, but have lost the expression of typical B-cell genes and acquired multiple other markers. Multiple signalling pathways are deregulated in Hodgkin–Reed–Sternberg cells, including NF (nuclear factor)-κB, JAK (Janus kinase)/STAT (signal transducer...
NHLs (non-Hodgkin’s lymphomas)

NHLs are common, especially in elderly patients, and have widely divergent clinical, immunological and molecular presentations. On the basis of these characteristics (aggressive compared with indolent, T-lineage compared with B-lineage), the prognosis and treatment are very different. Because of the divergent immunological, genetic and clinical phenotypes, no universal GEP for all NHLs is feasible.

Two review articles recently summarized the value of GEPIs in different types of NHL [21,22]. Overall, GEPIs can enable identification of new disease entities, development of new prognosticators, discovery of novel genes and pathways and understanding of pathogenetic mechanisms. Diffuse large B-cell lymphoma can be subdivided into at least two molecular subgroups [GCB (germinal centre B-cell-like type) and ABC (activated B-cell-type)]. These subgroups can be regarded as distinct clinicopathological entities. Patients with the GCB have a different set of genes transcribed than patients with the ABC. At the molecular level, primary mediastinal B-cell lymphoma can be defined as a subgroup of diffuse large B-cell lymphoma and shows some similarity with Hodgkin’s lymphoma. The subtype of mantle cell lymphoma is derived from B-lymphocytes and accounts for 5–8% of all cases of NHL. Clinically, mantle cell lymphoma has an intermediate to poor prognosis. Chromosomally, it has a well-defined marker [t(11;14)] that leads to overexpression of cyclin D1. At the molecular level, mantle cell lymphoma is homogeneous, but prognostically, it can be subdivided based on a proliferation gene signature. The prognostic models for diffuse large B-cell lymphoma and follicular lymphoma are influenced by signatures of the microenvironment. In follicular lymphoma, the immune response 1 profile [21] contains genes associated with T-cell activation and has a better prognosis. The immune response 2 profile contains transcripts derived from myeloid and monocytic cells and has a worse prognosis. Most cases of peripheral T-cell lymphomas are heterogeneous at the molecular level [21].

With the different types of NHL having largely different GEPIs, the miRNA profiles of various NHL types can also be expected to vary. In the GCB subtype of diffuse large B-cell lymphoma, but not in the ABC subtype, the amplification of the oncogenic miR-17-92 cluster and deletion of the tumour suppressor PTEN (phosphatase and tensin homologue deleted on chromosome 10) are recurrent [22]. For mantle cell lymphoma, Zhao et al. [23] used a platform containing 515 human miRNAs and performed miRNA expression profiling for 30 patients. The authors found that, compared with normal B-lymphocytes, 18 miRNAs were down-regulated (among them miR-29a/b/c, -142-3p/3p and -150) and 21 miRNAs (among them miR-124a and -155) were increased. They demonstrated that miR-29 has prognostic relevance (low values correlating with short survival). They also showed that miR-29 has functional relevance by inhibiting the CDK (cyclin-dependent kinase) 6 protein and miRNA by binding directly to the 3′-UTR. CDK6 is important in the pathogenesis of mantle cell lymphoma [23].

Ballabio et al. [24] recently established a miRNA expression profile for Sézary syndrome, an aggressive form of cutaneous T-cell lymphoma that in most cases has a poor prognosis. The majority of miRNAs expressed in this entity were down-regulated (104 out of 114). In their study, the authors examined 32 samples from 21 patients with Sézary syndrome, as well as 11 cases of mycosis fungoides. The profile for Sézary syndrome was clearly different from normal CD4-positive T-cells as well as from B-cell lymphomas. The authors also demonstrated that down-regulated miR-342 (which is intronically encoded) plays a role in pathogenesis by inhibiting apoptosis and described a novel mechanism of regulation (via binding of miR-199 to its host gene).

Another rare entity, NK (natural killer) cell neoplasms (leukaemias and lymphomas) are derived from NK cells, are generally positive for EBV (Epstein–Barr virus) and have an aggressive course. There are two subtypes (NK T-cell lymphoma/leukaemia (nasal type) and aggressive NK-cell leukaemia). So far, no disease-specific genes or cytogenetic markers have been identified. Yamanaka et al. [25] performed expression profiling in cell lines and patient samples and found that miR-21 and -155 are overexpressed in comparison with normal NK cells. This finding has functional relevance because, when both miRNAs were antagonized or reduced, PTEN, PDCD4 (programmed cell death 4) and SHIP1 (SH2 (Src homology 2)-domain-containing inositol phosphatase 1) became up-regulated, and the phosphorylated form of...
AKT (AKT Ser473) was decreased. Among the overexpressing NK cell lines and patient samples, the expression levels of miR-21 and -155 were inversely related.

Waldenström’s macroglobulinaemia is a disease of IgM-secreting lymphoplasmacytoid cells. Roccaro et al. [26] studied miRNA expression in this disease and found increases in miR-363, -206, -494, -155, -184 and -542-3p and a decrease in miR-9. According to this study, miR-155 regulated the proliferation and growth of the lymphoma cells in vivo and in vitro by inhibiting the MAPK (mitogen-activated protein kinase)/ERK, PI3K/AKT and NF-κB pathways. Six overexpressed miRNAs correlated with features of poor prognosis.

AML (acute myelogenous leukaemia)

In AML, treatment is now tailored according to cytogenetic aberrations. Bacher et al. [27] recently reviewed the contribution of genetic profiling to the diagnosis of AML. GEP can predict some genetic and molecular subgroups. In AML with normal cytogenetics, GEP can identify further biological and clinical subgroups. Further investigation of GEP may identify novel targets and subgroups.

miRNA profiling is a new technique and might reveal further distinctions in AML. Garzon et al. [28] studied the expression of miRNAs in a large number of newly diagnosed cases of AML. Several miRNAs showed differential expression between normal CD34+ cells and AML blasts. Certain cytogenetic and molecular types of AML were closely associated with their miRNA profile. Examples for such correlations are t(11q23), isolated trisomy 8 and FLT3 (FMS-like tyrosine kinase 3)-ITD mutations. Patients who overexpressed miR-191 and -199a had an unfavourable prognosis. This was confirmed in a multivariate analysis [28]. A follow-up study showed that restoring miR-29b in cell lines derived from AML and primary patient samples (by ectopic transfection) induced apoptosis and reduced tumourigenicity in a xenograft leukaemia model [29]. The miR-29 expression in these cells was inversely correlated with apoptosis genes, including MCL-1 (myeloid cell leukaemia-1). Older patients with AML respond better to the demethylating agent decitabine if they overexpress miR-29b (which targets DNA methyltransferases) [30]. In AML with MLL (myeloid/lymphoid or mixed-lineage leukaemia) gene rearrangements, the miR-17-92 cluster is frequently amplified, and the component miRNAs are overexpressed. These miRNAs may play a significant role in MLL-induced leukaemogenesis by inhibiting cell differentiation and apoptosis and regulating relevant target genes. MLL fusion proteins have stronger binding to the miR-17-92 locus than do the wild-type proteins [31].

CML (chronic myelogenous leukaemia)

CML has seen exciting technological advances over the last 40 years. From the recognition of a cytogenetic abnormality to bone marrow transplantation to PCR-guided treatment to the introduction of tyrosine kinase inhibitors, CML has been a model disease in modern haematology/oncology. Yong and Melo [32] recently reviewed the impact of gene profiling in CML. In earlier years, gene profiles revealed signalling pathways and resistance mechanisms in CML. Later studies showed increased Hox and Gata-2 transcription in CML progenitors, indicating increased self-renewal in CML. GEPs were used to understand the progression of the disease from chronic phase to blast crisis. During disease progression, PIASy (protein inhibitor of activated STAT) was found to be down-regulated and SOCS-2 (suppressor of cytokine signalling-2) up-regulated. Another use of GEPs was to predict either response to interferon or overall survival. Since the development of imatinib, GEPs have been used to differentiate responders from non-responders and quiescent CML stem cells from proliferating stem cells.

The discovery of miRNAs has now permitted investigators to study whether these molecules are involved in the pathogenesis, treatment response and progression of CML. Venturini et al. [33] studied the expression and treatment response of miRNAs in CML cell lines and patient samples. They found that polycistronic miR-17-92 is increased in the chronic phase of CML but not in blast crisis and is associated with bcr-abl and c-myc expression. When bcr-abl-positive cell lines were treated with the tyrosine kinase inhibitor imatinib (as well as with anti-bcr-abl and anti-c-myc interference), the expression of the miR-17-92 cluster was specifically down-regulated. According to this study, the overexpression of miRNAs in a CML cell line increased both proliferation and sensitivity to imatinib.

Bueno et al. [34] studied a fragile chromosomal region that encodes 12 % of all genomic miRNAs, including miR-203. This region is hypermethylated in several haematopoietic neoplasms, including CML. When miR-203 was re-expressed, levels of both ABL1 (c-abl oncogene 1; a target of miR-203) and BCR/ABL were reduced, and the proliferation of CML cells decreased. Therefore miR-203 was identified as a tumour suppressor in CML.

Agirre et al. [35] studied miRNA expression in mononuclear cells and CD34+ cells from CML patients in comparison with miRNA expression in cells from healthy controls. The investigators found an increase in miR-96 and a decrease in miR-10a, -150 and -151. The decrease in miR-10a did not depend on BCR-ABL1 activity and contributed to the increased proliferation of CML cells. USF2 (upstream stimulatory factor 2) was identified as a potential target of miR-10a. The clinical relevance of miR-10a and USF2 was demonstrated in a
group of 85 newly diagnosed patients with CML where the overexpression of USF2 was significantly associated with decreased expression of miR-10a. The same group of authors tentatively identified a group of 19 miRNAs that possibly predict resistance to imatinib [36].

**ALL (acute lymphoblastic leukaemia)**

ALL is a disease defined by its immunophenotype and additional chromosomal and molecular aberrations. Genomic profiling incorporating DNA copy number alterations and loss of heterozygosity has provided additional insights for prognosticating relapse and detecting genomic evolution [37]. Mi et al. [38] performed large-scale miRNA profiling comparing 18 cases of ALL and 54 cases of AML. Overall, 27 miRNAs were differentially expressed. Among these, miR-128a and -128b were significantly overexpressed, and let-7b and miR-223 were significantly down-regulated. These results were confirmed by real-time PCR. Even if only two miRNAs were taken as discriminators, the accuracy of discrimination was >95%. The overexpression of miR-128 was found to be associated with promoter hypomethylation [38]. Extending the use of miRNA profiling, Fulci et al. [39] compared 43 cases of B-lineage ALL with nine cases of T-lineage ALL and found that three miRNAs (miR-148, -151 and -424) were discriminators. Using Pearson correlation analysis, the investigators combined miRNA and mRNA expression profiles and found enrichment of miRNA:target pairs at negative correlations. Rainer et al. [40] identified two miRNAs (miR-223 and the miR-15~16 cluster) that are regulated by glucocorticoids.

**Multiple myeloma**

Because of the inherent heterogeneity of multiple myeloma, several groups have attempted to classify the disease based on its GEP. A French group characterized high-compared with low-risk myeloma based on a 15-gene risk score. At 3 years, the low-risk group had a survival rate of 90%; the high-risk group, 47%. Patients with high-risk myeloma were homogeneous in overexpressing genes of cell cycle progression and surveillance [41]. A group collecting bioinformatic data on GEPs from 877 individuals whose status ranged from normal plasma cells to monoclonal gammopathy of unknown significance to early and late multiple myeloma, described a signature that aimed at predicting prognosis and drug sensitivity. The evolution of the GEP from normal plasma cells was characterized by increased myc up-regulation and chromosomal instability [42]. More recently, a new attempt was made to classify multiple myeloma with GEPs based on 320 newly diagnosed patients from a Dutch–Belgian–German study. Hierarchical clustering identified ten distinct subgroups, six of which corresponded to clusters already described by a myeloma research group in Arkansas. One subgroup showed a myeloid signature [43].

The first miRNA profile of multiple myeloma, based on 49 cell lines and 16 CD138-selected patient samples, was published by Pichiorri et al. [44]. They found multiple changes with regard to both normal plasma cells and plasma cells from patients with monoclonal gammapathy of undetermined significance. Furthermore, when human myeloma cell lines were implanted into nude mice, treatment with miR-19a and -19b and with antagonists of miR-181a and -181b resulted in significant suppression of tumour growth. Löfler et al. [45] showed that the ectopic expression of miR-21 was sufficient to make IL-6 (interleukin-6)-dependent cell lines growth-factor-independent. Roccaro et al. [46] characterized an miRNA signature of multiple myeloma that somewhat differs from that of other authors (decreased expression of miR-15a and -16 and increased expression of miR-222, -221, -382, -181a and -181b). An Italian group attempted to correlate miRNA imbalances with the different cytogenetic subgroups [47]. For example, patients with t(4;14) showed overexpression of let-7e, miR-125a-5p and miR-99b. On the basis of these data, the investigators defined a network of putative interactions between miRNAs and their targets [47]. Zhou et al. [48] correlated the previously established Arkansas risk score for high-risk myeloma based on 70 genes with miRNA profiling and found a global elevation of miRNAs in high-risk disease. These cases also had increased proliferation. Among the 70 genes, expression of EIF2 (eukaryotic translation initiation factor 2)/AGO2 (Argonaute2), a master regulator of miRNA maturation and function, was increased. Silencing of AGO2 significantly decreased the viability of myeloma cell lines.

**miRNA PROFILING IN SOLID TUMOURS**

**Breast cancer**

Breast cancer is the most frequent cancer in women, with more than 200 000 cases diagnosed per year in the U.S.A. Early stages are curable by surgery and a combination of hormone treatment, chemotherapy and radiation. Late relapses may occur after many years. Late stages and metastatic disease respond to treatment, but are generally fatal. Among several GEP tests, two have been introduced commercially and are in wide use [49]. Mammaprint® is based on 70 genes, needs fresh or snap-frozen tissue and is performed with DNA microarrays. Oncotype DX® is based on 21 genes, needs formalin-fixed paraffin-embedded tissue and is performed with quantitative real-time PCR. The Mammaprint® assay initially assigned 30–40% of breast cancer patients to a low-risk category. The clinical value of this test is currently being studied in a randomized trial in women with zero to three positive lymph nodes to determine whether patients in the low-risk
GEP category can avoid adjuvant chemotherapy. In comparison with the Mammaprint® assay, the Oncotype DX® test assigns a higher percentage of women to the low-risk category, but has mostly been studied in patients with oestrogen-receptor-positive, node-negative disease.

In a multicentre international trial, the sensitivity or response of patients to two types of neoadjuvant chemotherapy was recently evaluated [50]. The end point of that study was pathological complete response. Using a 30-gene predictor, the sensitivity to one type of chemotherapy (T/FAC, T = paclitaxel, F = 5-fluorouracil, A = doxorubicin, C = cyclophosphamide) could be predicted with 38% accuracy, compared with only 8% for the other type of chemotherapy (FAC) [50].

The subtype of inflammatory breast cancer generally has an aggressive course and, despite multiagent chemotherapy, a poor clinical outcome. Six research groups attempted to establish GEPs for inflammatory breast cancer. Because of small sample sizes and clinical heterogeneity (including both basal and Her2-positive cases), no universal signature was found. Likewise, no signature predictive of treatment response or survival could be identified [51].

miRNA-based tests may bring an improvement for the classification of breast cancer and prediction of treatment response. At present, no miRNA profiles have been tested in larger patient groups or randomized studies. The first comprehensive study of miRNA profiles of breast cancer was published in 2005 [52]. The authors showed that miRNAs are expressed aberrantly in breast cancer. Using miRNA profiles, they were clearly able to discriminate normal breast tissue from breast cancer. The most dysregulated miRNAs were miR-125b, -145, -21 and -155. Some of the dysregulated miRNAs correlated with biopathological features such as oestrogen-receptor and progesterone-receptor expression, tumour stage, vascular invasion and proliferation. More recently, the same group of investigators showed that miR-205 (which is down-modulated in breast tumours compared with normal breast tissues) directly targets the HER3 receptor (important in a subtype of breast cancer) and thereby prevents the activation of the downstream mediator Akt [53]. In a different study, miR-221, -222 and -206 were introduced into oestrogen-receptor-positive cells. miR-221 and -222 increased proliferation, whereas miR-206 had an inhibitory effect. The authors concluded that miR-221 and -222 are involved in a regulatory loop with oestrogen receptor-α, possibly the transition to a receptor-negative state [54]. The reintroduction of miR-205 into the breast cancer cell line SKBr3 inhibited the clonogenic potential and sensitized the cells to the tyrosine kinase inhibitors gefitinib and lapatinib. In situ hybridization showed that the expression of miR-145 and -205 was restricted to myoepithelial and basal cells in normal mammary ducts and lobules, whereas their accumulation was absent or severely decreased in tumour specimens [55]. In contrast, expression of miR-21 was frequently increased in malignant cells. miR-145 is commonly reduced in human cancer. When Spizzo et al. [56] reintroduced miR-145 into breast cancer cell lines, they found a pro-apoptotic effect that is dependent on TP53 activation. miR-145 can also down-regulate oestrogen receptor-α protein expression through direct interaction with two complementary sites within its coding sequence. When TP53 (tumour protein p53) is activated, in turn, miR-145 expression is stimulated, thereby suggesting a death-promoting loop between miR-145 and TP53 [56]. miR-9 was recently shown to be involved in a breast cancer metastasis-promoting network involving E-cadherin and β-catenin [57].

Lung cancer

Often related to smoking, lung cancer is a common cancer (more than 200 000 new cases per year in the U.S.A.) with an overall poor prognosis. Both in early and late stages, better discriminators of treatment response and prognostication are needed. The value of genetic profiling in lung cancer was recently critically reviewed [58]. On the basis of 16 peer-reviewed studies and a detailed analysis of the studies’ sample preparation, validation with independent cohorts and statistical methods, the authors concluded that none of these profiling signatures is ready for clinical use.

miRNA profiling for lung cancer as a new method may improve the diagnostic and prognostic validity of gene profiling. Lebanony et al. [59] used a high-throughput microarray assay to distinguish lung adenocarcinomas from lung squamous cell carcinomas. As confirmed in validation cohorts as well as with formalin-fixed, paraffin-embedded tissues, miR-205 had a 96% sensitivity and a 90% specificity for distinguishing the two major types of non-small cell lung cancer. In a different study, 165 adenocarcinomas and 125 squamous cell lung cancers were analysed. These investigators used an oligoarray with 440 mature antisense miRNAs. Focusing on a different set of miRNAs as discriminators (miR-181a, -191, -107, -103 and let-7b), they could also differentiate adenocarcinoma from squamous cell lung cancer. Among male smokers with squamous cell carcinoma, the higher expression of five miRNAs (miR-25, -34c-5p, -191, let-7e, miR-34a) predicted longer survival [60]. A Japanese group performed expression profiling of 104 cases of non-small cell lung cancer (65 adenocarcinomas and 39 squamous cell carcinomas) comparing cancer tissues with normal tissues from the same patient. Five miRNAs (miR-155, -17-3p, let-7a-2, miR-145 and -21) with clearly different expressions were identified. A Kaplan–Meier analysis showed in a univariate analysis that high miR-155, and low let-7a-2 levels predicted short survival. A multivariate analysis confirmed the negative prognostic value for miR-155 [61]. Incoronato et al. [62] showed that miR-212 can sensitize lung cancer cells to apoptosis.
In most cases of lung cancer, miR-212 expression is low, whereas the expression of PED (phosphoprotein; a member of the death effector domain family with broad anti-apoptotic functions) is high. The authors showed that miR-212 is a negative regulator of PED. When cells sensitive to the molecule TRAIL (tumour-necrosis-factor-related apoptosis-inducing ligand) were treated with a synthetic antagonist (antagomir) to miR-212, they became resistant. In the reverse experiment, the ectopic expression of miR-212 induced apoptotic cell death in non-small cell lung cancer cells when treated in vitro with TRAIL [62]. Recently, a new miRNA that is oncogenic in lung cancer was identified [63]. Starting from lung cancer in transgenic mice, Liu et al. found that miR-136, -376a and -31 were prominently overexpressed. Among these, the antagonization of miR-31 suppressed tumour growth, suggesting causation. The oncogenic potential of miR-31 was substantiated when human lung cancer samples were studied. The authors also identified LAT52 (large tumour suppressor, homologue 2) and PPP2R2A (protein phosphatase 2, regulatory subunit B, α) as targets of miR-31 in mouse and human lung cancer. In another study, low miR-200c levels correlated with invasiveness in a panel of cell lines derived from non-small cell lung cancer. Likewise, in patient samples, low or absent miR-200c expression predicted an aggressive, invasive and chemoresistant phenotype [64].

Mesothelioma is histologically different from lung cancer, is often caused by asbestos and has a poor prognosis. Busacca et al. [65], on the basis of a small number of cases, established an miRNA signature for malignant mesothelioma and found that the reduced expression of miR-17-5p and -30c correlated with better survival in patients with the sarcomatoid subtype. In a larger series, Gee et al. [66] compared the miRNA expression pattern of malignant pleural mesotheliomas with lung adenocarcinomas. Using microarrays, they found a panel of miRNAs, including members of the miR-200 gene family, down-regulated in mesothelioma. These results were validated by RT-qPCR and, according to the authors, could serve as biomarkers for mesothelioma. They also predicted that the decrease of certain miRNAs interferes with Wnt signalling in mesothelioma.

**HCCs (hepatocellular carcinomas)**

A recent study compared GEPs between 20 Japanese patients with HCCs and normal or non-involved liver [67]. Eleven transcripts that were significantly increased in HCC were subjected to siRNA (small interfering RNA)-based screening, RT-qPCR and immunohistochemical analysis. Finally, four genes [AKR1B10 (aldo-keto reductase family 1, member B10), HCAP-G (non-SMC condensin I complex, subunit G), RRM2 (ribonucleotide reductase M2) and TPX2 (TPX2, microtubule-associated, homologue)] were chosen as candidate therapeutic targets. When these genes were knocked down by siRNA, the proliferation of HCC cells and the growth of HCC xenografts in immunodeficient mice were inhibited. Several groups have previously attempted to establish GEPs in HCCs [68]. As far as miRNAs are concerned, Ji et al. [69] analysed 455 Chinese patients who had undergone tumour resection between 1999 and 2003. Most of their patients had cirrhosis, and 90% were carriers of the hepatitis B virus. The expression of miR-26 was strikingly lower in tumours compared with non-tumorous liver tissues from the same patients. Tumours with reduced miR-26 expression had a distinct transcriptomic pattern (activation of the IL-6 and NF-κB pathways). Interestingly, women had a higher expression of miR-26 in non-tumorous liver tissues, and patients with a low expression of miR-26 had shorter overall survival but responded better to interferon than patients who had high expression of miR-26. A different group of investigators performed miRNA expression profiling on HCC in patients from France and compared these data with data for normal livers, cirrhotic livers and cell lines derived from HCC [70]. A total of 12 miRNAs (among them miR-21, -221/222, -34a, -519a, -93, -96 and let-7c) were correlated with tumour progression. miR-221 and -222 were the most up-regulated miRNAs and were shown to target the CDK inhibitor p27 in vitro and stimulate cell growth in vitro. The effects of miR-221 were also reproduced in a mouse model of HCC. The molecule DDIT4 (DNA damage-inducible transcript 4), a modulator of the mTOR (mammalian target of rapamycin) pathway, was also identified as a target of miR-221 [70]. Gramantieri et al. [71] identified Bmf as a target of miR-221 in HCC. In their study, high expression of miR-221 correlated with tumour multifocality and reduced time to recurrence after surgery. The antagonization of miR-221 increased cell death and susceptibility to apoptosis. A different miRNA, miR-199a-3p, was decreased in HCC; it was recently shown to regulate mTOR and c-met. When the low levels of miR-199a-3p were restored to normal levels, the cells were arrested in the G1-phase of the cell cycle, became susceptible to hypoxia and became sensitized to doxorubicin [72]. A group from the United States with partners in Spain and Italy recently described miR-517a as a novel oncogenic miRNA in HCC [73]. The authors discriminated three clusters of HCC (Wnt-related, IFN (interferon)-related and proliferation-associated). In the subtype of proliferation-associated HCC, they identified miR-517a (located on chromosome 19q13.42) as oncogenic.

**Colon cancer and other gastrointestinal malignancies**

Adenocarcinoma of the colon is a common cancer, with more than 100 000 cases diagnosed per year in the U.S.A. Genetic profiling has been found useful for
Several groups have studied miRNAs in ovarian cancer [81]. Bhattacharya et al. [82] recently showed that miR-15a and -16 are down-regulated in ovarian cancer. These miRNAs target the 3′-UTR of Bmi-1 and had an inverse correlation with the expression of the Bmi-1 protein in ovarian cancer samples. In addition, when miR-15a or -16 was overexpressed, the levels of Bmi-1 decreased, and clonal growth and proliferation slowed [83].

Melanoma
Malignant melanoma (in advanced stages) is an aggressive tumour that rarely responds to chemotherapy. However, some cases respond to immunotherapy, and more recently, molecular targeted therapy is being developed. Consequently, investigators have tried to determine which patients respond to immune interventions and have developed a GEP for this purpose [84]. In the MAGE-A3 (melanoma-associated antigen 3) immunotherapy trial, the group with a favourable clinical outcome had high expression of the chemokines CCL5 (CC chemokine ligand 5), CXC9 (CX chemokine 9) and CXCL10 (CX chemokine ligand 10). In a recent study on melanocytes, melanoma cell lines and 20 melanoma biopsy specimens, miR-146 and -155 were increased only in patient samples [85]. miR-200c was consistently down-regulated in melanocytes, melanoma cell lines and patient samples. miR-205 and -23b were decreased only in patient samples.

In pathway analysis, MITF (microphthalmia-associated transcription factor) was identified as a deregulated target. In a study of patients with melanoma that had metastasized to lymph nodes, Caramuta et al. [86] compared cases with and without BRAF (v-raf murine sarcoma viral oncogene homologue B1) mutations; cases with BRAF mutations underexpressed miR-193a, -338 and -565. Overall, in this small series of cases, low expression of miR-191 and high expression of miR-193b were associated with poor prognosis. Recently, miR-196a, which is strongly decreased in melanoma, was described as a regulator of HOX-B7 (homeobox B7) and BMP4 (bone morphogenetic protein 4) in malignant melanoma [87].

Glioblastoma
Vital et al. [88] recently classified 40 glioblastomas based on their GEPs and could clearly distinguish high- and low-grade tumours. Among cases of glioblastoma multiforme, the investigators identified three subgroups that were closely associated with the cytogenetic profile of their ancestral tumour cell clones. The three subgroups also were characterized by EGFR (epidermal growth factor receptor) amplification, isolated trisomy 7 and more complex karyotypes. In a recent study analysing the expression of 365 mature miRNAs in malignant gliomas, 16 miRNAs were identified as associated with malignant progression. Among them, miR-196a and
-196b showed the most significant differences between tissues at baseline and after progression. Patients with high miR-196 levels also had the shortest survival [89]. Kim et al. [90] performed an integrative analysis showing that miR-26a is located at an amplicon (12q13.3–14.1) that is frequently amplified in glioblastoma. The authors identified PTEN, RB1 (retinoblastoma 1) and MAPK2/MEKK2 (MAPK kinase kinase 2) as targets of miR-26a in glioblastoma. miR-26a alone transformed cells and promoted glioblastoma growth in the mouse brain. Patients with miR-26a amplification (and CDK4 amplification) had shortened survival. Cortez et al. [91] recently reported that miR-29b and -125a regulated podoplanin in glioblastoma and suppressed invasiveness. Both miRNAs were significantly down-regulated in glioblastomas as well as in CD133-positive glioblastoma stem cells. The transfection of miR-29b in tumour cells reduced proliferation and induced apoptosis [preferentially in glioblastoma cells with wild-type TP53 (tumour protein p53)]. miR-21 was shown to target a network of tumour-suppressive pathways in glioblastoma [p53, TGF-β (transforming growth factor-β) and mitochondrial tumour-suppressive genes] [92].

Other solid tumours

In paediatric germ cell tumours, most miRNAs are down-regulated. However, the components of miRNA clusters miR-371, -373 and -302 are overexpressed regardless of tumour type, tumour site or patient age group. By a computer algorithm (Sylamer), this miRNA expression pattern was correlated with the expression pattern of miRNAs in germ cell tumours. The algorithm showed that the hexamer GCACCTT (complementary to the 2–7 nucleotide miRNA seed sequence of both miRNA clusters) was the only sequence significantly enriched in the 3′-UTR of miRNAs down-regulated in all types of germ cell tumours [93].

For head and neck cancer, a recent study examined 51 formalin-fixed samples from patients with squamous carcinomas [94]. The authors detected 38 miRNAs that were differentially expressed between cancerous and normal tissues. No significant differences were observed between laryngeal, oropharyngeal or hypopharyngeal cancers. In addition to previously described changes, increases in levels of miR-423, -106b, -20a and -16, as well as a decrease in miR-10a levels, were found. In cell lines derived from squamous head and neck cancer, the transfection of miR-375, as well as knockdown of the miR-106b-25 cluster, led to decreased proliferation and clonal growth. miR-451 discriminated samples from patients who had relapses and patients who did not.

Pancreatic carcinoma has a poor prognosis and limited treatment possibilities. Giovanetti et al. [95] investigated tumour samples from 81 patients with pancreatic ductal adenocarcinoma and found that high miR-21 expression (in microdissected tumour samples) correlated with short survival both for patients who received adjuvant treatment and those who had metastatic disease. The overexpression of miR-21 correlated with gemcitabine resistance. When pancreatic tumour cells were transfected with the precursor of miR-21, further resistance to apoptosis (caused by gemcitabine) was induced, and expression of MMP (matrix metalloproteinase)-2/MMP-9, as well as vascular endothelial growth factor, was up-regulated. Greither et al. [96] studied miR-155, -203, -210 and -222 in microdissected pancreatic carcinoma samples and found that elevated expression of each miRNA increased the risk of death. In combination (if all four miRNAs were overexpressed), the risk of death from cancer was increased 6.2-fold.

Prostate cancer is a common cancer in older men with a wide spectrum of disease dynamics (from indolent to highly aggressive). miRNA profiling may be helpful in diagnostic and prognostic discrimination. Ambs et al. [97] studied 60 primary prostate cancers and 16 non-tumorous prostate tissues. They found dysregulation of miRNA processing and clear-cut up-regulation of the miR-106b-25 cluster and of miR-32. They identified new targets of miR-106b (E2F1 (E2F transcription factor 1) and p21/WAF1), of miR-32 (Bim) and of miR-1 (exportin-6 and protein tyrosine kinase 9) in prostate cancer. Szczyszyba et al. [98] studied the miRNA profile of prostate cancer by deep sequencing and found 33 miRNAs up- or down-regulated more than 1.5-fold compared with normal tissues. These results were confirmed by Northern blotting and RT-qPCR. Myosin VI, which is up-regulated in primary prostate cancers, was identified as a target of miR-143 and -145. A mutation of the potential binding sites for miR-143 and -145 resulted in a loss of responsiveness to the corresponding miRNAs. Confirming these data, a different group found that miR-221 is progressively reduced in aggressive and metastatic prostate cancer [99]. miR-34a was recently shown to inhibit or target prostate cancer stem cells [100]. The authors showed that miR-34a (a target of p53) is underexpressed in prostate cancer stem cells. By enforcing the expression of this miRNA in bulk or CD44-positive prostate cancer cells, they could inhibit clonogenic growth, tumour regeneration and metastasis. When the adhesion molecule CD44 was knocked down, similar effects were seen.

Pallante et al. [101] performed genome-wide expression profiling for 30 thyroid papillary carcinomas and compared the miRNA expression with normal thyroid tissues. They found an aberrant profile (significant increase of miR-221, 222 and -181b in tumours) that differentiated cancer from normal. A similar pattern was also observed in fine-needle aspirates, which turned out to be thyroid cancer and in transformed mouse cell lines. As for anaplastic thyroid cancer, Visone et al. [102] found a different profile with specific miRNAs (among them miR-30d, -125b and -30a-5p) down-regulated compared
with normal thyroid tissue. These data were obtained using a chip microarray but confirmed by Northern blots, RT-qPCR and in situ hybridization. The cell growth of a thyroid cancer cell line was inhibited when the cells were transfected with miR-125b or -26a.

**Carcinoma of unknown primary**

Molecular profiling is being used to assign a histogenetic origin in tumours classified as ‘carcinomas of unknown primary’ and to correlate the results with clinical outcome [103]. Using paraffin-embedded tissues and RT–PCR for ten genes in 104 patients, a tissue of origin was assigned in 61 % of the cases in which the test could be used successfully. The tumours most commonly identified were cancers of the lung, pancreas and colon. In most patients, the assigned tissue of origin was compatible with clinicopathological features and response to treatment.

miRNA profiling may be helpful in further discriminating the histogenesis and best treatment for carcinomas of unknown primary. In an attempt to classify tumours based on their miRNA profiles, Rosenfeld et al. [104] measured the expression of miRNAs levels in 400 paraffin-embedded and fresh-frozen samples by a custom-made microarray. Overall, 22 different types of tumour tissues were represented. The authors built a transparent classifier based on a decision tree using 48 miRNAs and data from 253 samples. Most samples could be classified with high confidence. In an independent verification data set of 83 samples, the overall high-confidence accuracy was 89 %. This study was recently updated using 356 formalin-fixed, paraffin-embedded tumour samples of known origin and showed 85 % concordance with the reference diagnosis [105]. It will be interesting to see what an miRNA-based test (in combination with other test methods) can contribute to the diagnosis of carcinomas of unknown primary.

**COMMON THEMES IN THE STUDY OF miRNAs IN CANCER AND GENETIC RISK ASSESSMENT**

Common themes across cancer types for the action or influence of miRNAs on cancer phenotypes are that they confer resistance or, in other cases, susceptibility to apoptosis and that they confer growth factor or stroma dependence or independence. Some miRNAs promote tumour growth (oncomirs); other miRNAs antagonize tumour growth (tumour-suppressor miRNAs). As discussed above, some miRNAs promote metastasis; others promote angiogenesis. miRNAs may also be involved in the de novo or induced resistance to chemotherapy drugs. Thus overcoming drug resistance might be achieved by antagonizing or substituting miRNAs (for a review about drug resistance and miRNAs, see Ma et al. [106]). miRNAs are involved in the inherited predisposition to cancer, as exemplified by CLL [10]. Beyond CLL, subtle differences in miRNA expression and regulation may influence the risk for many cancers. In support of this hypothesis, Nicoloso et al. [107] analysed SNPs (single nucleotide polymorphisms) in target genes known to influence the risk for breast cancer and found two SNPs that modulate gene expression [miR-187 interacting with rs1982073-TGFB1 and miR-138 interacting with rs1799782-XRCC1 (X-ray repair complementing defective repair in Chinese-hamster cells 1)]. In a population-based study, Christensen et al. [108] found that a polymorphism in mature miR-196A2 reduced the risk of developing head and neck squamous carcinoma. In addition, in a subset analysis, homozygous carriers of the polymorphism with pharyngeal cancer had a worse prognosis compared with individuals with wild-type and heterozygous genotypes. Heterozygosity of a SNP (G/C) within the precursor of miR-146a was found to be a predisposing factor for papillary thyroid cancer. Interestingly, heterozygotes differed from homozygotes by producing three types of mature miRNAs (one from the active strand and two from the passenger strand). These three miRNAs correlated with widely different transcriptomes, which might explain the different susceptibility to thyroid cancer [109]. Bao et al. examined 61 common SNPs in miRNAs and their target genes in a group of 601 men with advanced prostate cancer who were treated with hormone ablation [110]. The authors found that four were associated with disease progression, seven were associated with death from prostate cancer and four were significant predictors of death from all causes. In a multivariate analysis, which included clinicopathological features, most SNPs remained significant for disease progression and death from prostate cancer. For patients with more than one unfavourable genotype, the risk of disease progression and death from prostate cancer increased further. Liang et al. investigated 226 SNPs in miRNA-processing genes and miRNA-binding sites in 339 women with ovarian cancer and 349 healthy controls [111]. They discovered 13 polymorphisms significantly associated with risk for ovarian cancer. Two linked SNPs (rs2740351 and rs7813) in the GEMIN4 [gem (nuclear organelle)-associated protein 4] gene had the highest association with risk. As far as the outcome is concerned, 24 SNPs were associated with overall survival and 17 with treatment response. Of note is that a rare homozygous genotype of the PDGFC (platelet-derived growth factor C) gene (rs1425486) correlated with the shortest overall survival and the worst treatment response. In addition, a gene dosage effect was observed with a worse prognosis in patients who had several adverse genotypes. In malignant melanoma, a variant in 3′-UTR of the KIT oncogene was described with correlates with a more than 4-fold increased risk of acral melanoma [112]. This variant...
results in a mismatch in the seed region of a miR-221 complementary site and potentially in an increased expression of KIT.

A NEW TREND IN TRANSLATIONAL ONCOLOGY: SOLUBLE miRNAs

Serum or plasma is easily available and can be used for screening and follow-up purposes. The search for serum biomarkers of cancer is ongoing. Only a few serum biomarkers are established for the diagnosis of cancer: α-fetoprotein for HCC, β-human chorionic gonadotropin for germ cell tumours and prostate-specific antigen for prostate cancer.

miRNAs in serum or plasma have been investigated in several tumour types. In one study, it was found that normal human plasma contains stable miRNAs that are protected from endogenous RNase activity [113]. The authors demonstrated that they could measure miR-141 derived from prostate xenografts and use it to distinguish prostate cancer patients from healthy controls. In a different study, levels of two miRNAs (miR-17-3 and -17-92) were significantly increased in the plasma of patients with colorectal cancer in comparison with levels in healthy controls. The levels of these markers decreased after surgery. The authors foresee, if specificity is confirmed, that such plasma-based biomarkers could be used for screening for colorectal cancer [114]. The miRNAs miR-20a and -20b are frequently overexpressed in pancreatic cancer. In a recent study, most patients with pancreatic cancer had elevated serum levels of these miRNAs in comparison with normal controls. However, a similar elevation was also observed in patients with chronic pancreatitis [115]. When investigators applied plasma-based miRNA profiling to non-small cell lung cancer, they found a decrease in serum levels of three miRNAs compared with normal controls (let-7f, miR-20b and -30e-3p), two of which were associated with a poor outcome [116]. In plasma from patients with gastric cancer, the concentrations of four miRNAs (miR-17-5p, -21, -106a and -106b) were significantly higher than in controls, whereas the concentration of let-7a was lower. According to the authors, the elevated levels of plasma miRNAs derived from the tumour in most cases and decreased after surgery [117]. All these studies appear promising but should be repeated in larger patient groups and with more controls before routine use in the diagnosis and follow-up of cancer can be considered.

THERAPEUTIC POTENTIAL OF miRNAs

Beyond the interesting data derived from profiling, an important question is whether miRNAs can be used directly for the treatment of patients with cancer. Treatment could be effected by either transfecting tumour-suppressor miRNAs or antagonizing oncogenic miRNAs. The relevant miRNAs could be introduced into the systemic circulation or injected into part of the body (for example, into the peritoneal cavity or into a limb) or directly into a tumour. Alternatively, the therapeutic could be introduced into stem or progenitor cells, which would subsequently be used for transplantation. If miRNAs have antitumour activity in vivo, combination with standard approaches (chemotherapy, immunotherapy, radiotherapy) is conceivable. In light of the theory that current chemotherapy or radiation fails in many cases because tumour stem cells persist, a new approach would be to target treatments directly to cancer stem cells. A major impact on cancer could be made if tumour-suppressor miRNAs or antagonists to oncogenic miRNAs could be targeted to cancer stem cells or used in combination with classic treatments for cancer.

Before an miRNA-based treatment can be considered in humans, extensive preclinical studies for safety and toxicity are necessary. At present, few reports of animal models using miRNAs for the treatment of cancer have been published. A commonly used model for assessing the growth of human tumours is the nude mouse. Such studies (xenograft models using immunosuppressed mice) showed that myeloma cell lines (U266 and JNN3) transfected with miR-19 and -181 antagonists resulted in significant tumour suppression in comparison with cells transfected with an empty vector [44]. Conversely, when a glioma cell line (U87) expressing a low level of miR-34 was injected into the brains of immunosuppressed mice, the concomitant transfection of the precursor of miR-34 led to a drastic reduction of tumour growth [118]. In the brain tumour cells transfected with miR-34a, the targets c-met and Notch were down-regulated.

In several other disorders (hypercholesterolaemia and hepatitis), promising preclinical models using miRNAs were published. Mir-122 is expressed in liver in a tissue-specific manner and is essential for the accumulation of the hepatitis C virus. In a primate model of chimpanzees chronically infected with hepatitis C, the animals were treated with locked nucleic-acid-modified oligonucleotides complementary to miR-122. In subsequent liver biopsies, miR-122 levels were depressed, and the systemic hepatitis C viral load was decreased [119]. The virological response was long-lasting without emergence of resistant mutants. At the same time, the liver histological status improved, and interferon-regulated genes were downmodulated.

miR-34a is considered a tumour suppressor miRNA because it has reduced expression in several common types of cancer, including lung cancer. Wiggins et al. [120] synthesized an miR-34a mimic and a lipid-based delivery vehicle. Few side effects were observed when this molecule was administered in a mouse model of lung cancer; in particular, there was no elevation
of systemic cytokines or liver enzymes. The intratumoural or systemic administration of miR-34a delayed or blocked tumour growth. This therapy was accompanied by an accumulation of the miRNA in the tumours and a down-regulation of direct targets of miR-34a, such as CDK4 and c-met [120].

Cells from HCC generally have low expression of miR-26a. When miR-26a was administered systemically in a mouse model of HCC using an adenovirus-associated vector, the development of HCC was prevented, and tumour-specific apoptosis was induced without overt toxicity [121]. In a mouse model of metastasis involving the injection of human breast cancer cells, overexpressing miR-31 prevented or delayed lung metastasis [122]. In a prostate cancer model, the injection of miR-16 with atelocollagen inhibited the growth of prostate tumours in bones [123]. Taulli et al. [124] showed that the substitution or reexpression of miR-206 could lead to differentiation of human rhabdomyosarcoma cells. They demonstrated that the met tyrosine kinase receptor is an essential target for the effects of miR-1 and -206. Similar approaches might lead to a differentiation therapy for human cancer. If the administration of miRNAs and antagonirs can be shown to be safe and effective in patients with cancer, then a major impact on modern oncology can be foreseen.

CONCLUSIONS AND OUTLOOK

Genetic profiling based on mRNA has found a limited role in the risk assessment of breast cancer and is used in a research setting in leukaemia, lymphoma and multiple myeloma. miRNAs regulate gene expression and translation and are important in the initiation and progression of cancer. Genetic profiling based on miRNAs holds many advantages over mRNA-based assays (better classification of tumours, prediction of treatment response, stability in plasma and paraffin-embedded tissues), but at present, more research and standardization are necessary. miRNAs and profiles based on miRNAs may also become useful in gene therapy for cancer. A brief overview and comparison of GEP and miRNA profiling is given (Table 1).

<table>
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<th>Characteristic</th>
<th>GEP</th>
<th>miRNA profiling</th>
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<tr>
<td>Source of tissue</td>
<td>Fresh or snap-frozen, (paraffin-embedded), normal tissue, tumour tissue</td>
<td>Fresh or snap-frozen, paraffin-embedded, normal tissue, tumour tissue, plasma, (serum)</td>
</tr>
<tr>
<td>Number of genes for study</td>
<td>Thousands</td>
<td>Hundreds</td>
</tr>
<tr>
<td>Clinical use</td>
<td>Yes (risk assessment in breast cancer, tissue source in carcinoma of unknown primary)</td>
<td>Potential (classification, prognostication, to be validated by prospective studies)</td>
</tr>
<tr>
<td>Research use</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Classification of cancer</td>
<td>++</td>
<td>+++</td>
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