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Hepatoblastoma and microRNA-483 Two Forms and One Outcome

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Abstract

Hepatoblastoma (HB) is the most common liver cancer in infants younger than 3 years. Its onset has been associated with other genetic syndromes and some genetic and biochemical markers has been identified recently in this neoplasia. Nevertheless the patients have a poor prognosis and the resection or transplantation remains the only effective therapeutic approach. The identification of non-invasive markers may represent an innovative approach and may contribute to a more accurate histological classification of this tumor. We previously demonstrated that some microRNAs are helpful in discriminating HB from hepatocellular carcinoma. In this study, we describe the involvement of the two forms of microRNA-483 (-3p and -5p) in a selected cohort of HB patients who underwent surgical resection or liver transplantation. Differently from other liver diseases we observed that the quantitative expression of the two forms did not significantly changed among patients. Furthermore, 3p/5p ratio was different between HB and non-HB samples, being positive in the latter and negative in HB samples. Influence of concomitant treatments in the expression of miR-483 (i.e. chemotherapy, and immunosuppressive drugs) was also evaluated and no changes were found in the follow-up. In conclusion the expression and function of miR-483-3p/5p in HB still remains unclear and further studies are needed to elucidate the possible mechanisms that regulate the different strand selection between the two forms of microRNA-483 in patients affected by HB. We deem that the analysis of microRNA-483 different forms could be useful for the molecular identification of HB patients and the discrimination with non-HB patient.

Keywords: microRNAs; Hepatoblastoma; Liver pediatric tumor; Biomarker

Abbreviations: Ctr, Control; FAP, Familial Adenomatous Polyposis; APC, Adenomatous polyposis coli; HB, hepatoblastoma; HCC, hepatocellular carcinoma; Igf2, Insulin-like Growth Factor 2; WNT, Wingless; miR, MicroRNA; SIOPEL, Société Internationale d'Oncologie Pédiatrique – Epithelial Liver Tumor Study Group; NA, not assessed; Pt, Patient.

Introduction

Hepatoblastoma (HB) is a rare malignant embryonal tumour of the liver that occurs in young infants with a median age at diagnosis of 18 months and its annual incidence is below 0.15 patients per 100,000 population under 15 years of age [1]. HB accounts for 1% of new cancer diagnoses in childhood and is the most common childhood liver cancer [2]. HB etiology is still unknown, most cases are sporadic whereas several genetic syndromes are associated with approximately 15% of cases and a close association with developmental syndromes such as the Beckwith-Wiedemann Syndrome (BWS) and Familial Adenomatous Polyposis (FAP) has been widely described [3,4,5].

Several distinct histological subtypes of HB have been identified. This heterogeneous tumor spectrum reflects different patterns of embryonal liver development and maturation, indicating a developmental origin for HB and a huge variation in the clinical outcome [6]. Five biomarkers, β-catenin, E-cadherin, Cyclin D1, Ki-67 and alpha-fetoprotein, have been widely described in HB onset and progression [6-11]. Among these markers, β-catenin is deeply involved in Wnt/β-catenin pathway playing a central-key role in hepatic development, regeneration, and tumorigenesis. Notably HB presents the highest rate (50–90%) of β-catenin mutations [12]. Nevertheless, Wnt and β-catenin expression are not always sufficient to characterize the differences between tumoral and non-tumoral in HB samples [13]. As an example, despite Adenomatous polyposis coli (APC) or

β-Catenin mutations are present in all colon cancer cells, WNT pathway is not always activated. Furthermore, high WNT activity and nuclear β-Catenin expression were often found to the tumor margin area, reflecting an intratumoral WNT signaling heterogeneity [14,15]. This phenomenon has been termed the "β-Catenin paradox" and led to the discovery that other specific modulators (i.e. hepatocyte growth factor and mitogen-activated protein kinase) may modulate WNT signaling activity with APC or β-Catenin mutations, thus contributing to a WNT mediated tumor progression [16-20].

HB is still burdened by a poor prognosis and invasive intervention, and the only effective therapeutic approaches are the liver resection or transplantation with a significant rate of unfavorable outcomes. The identification of HB molecular biomarkers, collectable from patients through blood or organic fluids, may represent a less invasive approach useful not only for the histological characterization but also for the comprehension of the biological pathways involved in HB tumorigenesis. This would lead to an increased knowledge on its pathogenesis and it might influence the overall survival rates [6].

MicroRNAs (miR) are small non-coding RNAs that influence the pathogenesis of human diseases, including cancers, by binding their

target messenger RNAs preventing the protein translation [21]. From the functional point of view they can either act as oncogenes or tumor suppressors. Large-scale approaches have been shown that several miR differentially expressed in liver tumors are involved in the progression of the disease [13, 22-26].

We previously demonstrated that five miRs (miR-214, miR-199a, miR-150, miR-125a, and miR-148a) are useful in discriminating HB from hepatocellular carcinoma (HCC), thus representing a new class of markers for the classification of pediatric liver tumors [13]. Preliminary data from gene expression profile in this work also highlighted the involvement of miR-483.

MiR-483 gene is located on genomic chromosome at 11p15.5 in the second intron of Insulin-like Growth Factor 2 (Igf2) and produces two mature different forms: miR-483–3p and miR-483–5p, differentially involved in liver pathologies [27,28-34]. MiR-483-5p and miR-483-3p were identified from a human embryonic liver. Reports have suggested that some intragenic miRNAs co-express and cooperate with their host genes, but that other miRNAs do not. Igf2 over-expression promotes proliferation and carcinogenesis during the progression from liver fibrosis to HCC. In addition, miR-483 is up-regulated in approximately half of human tumors including adrenocortical carcinoma and HCC, and its oncogenic targets (PUMA, CTNNB1, IGF1R) have been identified [35,36].

The biogenesis of miRs involves the stepwise cleavage of a long primary miR (pri-miR) transcript in a small hairpin-shaped RNA called pre-miRNA. About one third of miR genes are located in the introns of protein-coding genes; these are overwhelming found on the sense strand, implying a linkage of miR and host mRNA transcription. Once in the cytoplasm, the pre-miR hairpin is recognized and cleaved within its stems. This yields paired ~22 nt RNAs, known as the miR-5p/miR-3p duplex formed by two strands, one of which is degraded. Recent studies indicate that the dominant forms of a miRNA differs amongst tissues, times of development, and between species, suggesting the existence of other mechanisms for controlling the selection of mature miR [37].

In the present study we evaluated the content of the two different forms of miR-483 in a selected cohort of HB patients who underwent surgical resection or liver transplantation. By the analyses of the biopsies from surgical tissue samples we found that the relative concentration of the miR-483-5p was higher than the -3p one in HB patients. Conversely, in non-HB patients, the relative concentration of the -3p form was predominant. Furthermore, we demonstrated that the two forms were also detectable in serum samples, suggesting the potential use of miR-483 as noninvasive biomarkers for HB. Finally and most importantly, by analyzing patients in the follow-up, we found the same pattern of expression of the two forms, leading to the hypothesis that, although the exact cause of liver cancer is still unknown, some genetic factors may contribute to miR483-5p expression independently by the healthy status of a patient after tumor treatment.

Materials and Methods

Case selection

A total of 14 unrelated confirmed cases of HB (Patient, Pt) were included in the study. They all received surgical treatment (3 liver resection and 11 liver transplantation) at the General Surgery Department and Liver Transplant Center of the AOU Città della Salute e della Scienza di Torino, Italy. Written informed consent was obtained from parents of all patients for these studies.

Two patients deceased in the follow-up following transplantation, while one resected patient was lost to follow-up.

Seven tissue samples were obtained from surgical specimens immediately after tumor removal. Ten serum samples were collected from HB patients in the follow-up after surgical treatment (Table 1). In three cases (Pt1, Pt3, Pt7) we analyzed both tissue and serum. With regards Pt4 we included an RNA extracted from a non-neoplastic part of a liver explanted for HB (Ctr3).

In all cases, histopathological diagnosis of HB was obtained from specimens of percutaneous ultrasound-guided biopsy. Pre-operative staging was based on computed tomography imaging, according to PRETEXT criteria. All patients underwent neo-adjuvant chemotherapy, according to Société Internationale d'Oncologie Pédiatrique – Epithelial Liver Tumor Study Group (SIOPEL) protocols [9].

Non-HB samples (Control, Ctr) were also included in the study. As regard tissue analyses, we used two RNAs from normal individuals: Ctr1 sample was a pooled RNA from a fetal liver and Ctr2 was a pooled RNA from adult liver. Both Ctr1 and Ctr2 were commercially purchased by Clontech.

Regarding serum samples, we included 14 non-HB individuals in the study. In particular, Ctr4 to Ctr13 had undergone liver transplantation for non-neoplastic diseases (Familial cholestasis, Biliary atresia, Sclerosing cholangitis, Vascular anomaly, Urea cycle disorder); Ctr14 to Ctr17 were healthy donors. Tacrolimus was administered to Ctr4 to Ctr13 controls as first line immunosuppressor (Table 1S – supplementary data).

All data were collected from medical record. For each patient, histopathological diagnosis was confirmed from two different pathologists by revision of the liver biopsies. Written informed consent for biological studies was obtained also from all controls or their families.

microRNA isolation from tissues

Total RNA from HB tissues was isolated using TRIzol reagent (Invitrogen, Carlsbad,CA, USA) following the manufacturer's instructions. Two commercial RNA extracted from a fetal (Ctr1) and adult liver (Ctr2) tissue were also used as control RNA in order to test our hypotheses.

microRNA isolation from serum

The total RNA, including miRs, was extracted from serum using the miRNeasy Serum/Plasma Kit (Qiagen, Hilden, Germany). Two hundred microliters of serum were mixed with denaturing buffer in the volumes described in the manufacturer's protocols. The homogenate was incubated at room temperature for 5 min. Subsequently, the manufacturer's protocols were followed for RNA extraction. Total RNA was eluted into 14 μl of nuclease-free water. The RNA concentration was measured using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

TaqMan real-time PCR

To specifically evaluate mature miRs, TaqMan® MiRNA assay kits were purchased from Life Technologies® (Foster City, CA) to detect miR-483-3p and miR-483-5p. It is a two-step protocol requiring reverse transcription with a miR specific primer, followed by real-time PCR with TaqMan probes.

Mature miR expression was assayed by TaqMan MicroRNA assay (Life Technologies®) specific for miR-483-3p and miR-483-5p.

Sample	Sample type	Gender	Age at OLT (mths)	preCT state	CT treatment	Days of follow up	Diagnosis
Pt1	T + S	F	20	Pretext III, P1	SIOPEL IV	1240	Hepatoblatoma
Pt2[†]	T	M	23	Pretext III, P1	SIOPEL IV	2	Hepatoblatoma
Pt3	T + S	F	31	Pretext IV, V1	SIOPEL IV	1410	Hepatoblatoma
Pt4[†]	T	F	37	Pretext III	SIOPEL III	150	Hepatoblatoma
Pt5[°]	T	M	15*	Pretext II	SIOPEL III	2160	Hepatoblatoma
Pt6[°]	T	M	143*	Pretext II	SIOPEL III	lost	Hepatoblatoma
Pt7	T + S	F	8	Pretext III, P1 V2	SIOPEL IV	1021	Hepatoblatoma
Pt8[°]	S	M	17*	Pretext II	SIOPEL IV	345	Hepatoblatoma
Pt9	S	F	34	Pretext IV	SIOPEL III	2550	Hepatoblatoma
Pt10	S	M	30	Pretext IV, P1, V2	SIOPEL III	2023	Hepatoblatoma
Pt11	S	M	14	Pretext IV	SIOPEL IV	471	Hepatoblatoma
Pt12	S	M	63	Pretext IV	SIOPEL II	4747	Hepatoblatoma
Pt13	S	F	32	Pretext IV	SIOPEL IV	1266	Hepatoblatoma
Pt14	S	M	8	Pretext III	SIOPEL III	1873	Hepatoblatoma
Ctr1[§]	T	M/F	20-38 weeks	NA	NA	NA	Normal pooled human fetal livers
Ctr2[§]	T	M	288-768	NA	NA	NA	Normal pooled livers
Ctr3[†]	T	F	37	Pretext III	SIOPEL III	150	non-neoplastic part of a liver explanted for HB
Ctr4	S	M	37	NA	NA	559	Familial cholestasis
Ctr5	S	M	8	NA	NA	849	Biliary atresia
Ctr6	S	M	192	NA	NA	1415	Sclerosing cholangitis
Ctr7	S	F	95	NA	NA	3292	Vascular anomaly
Ctr8	S	F	5	NA	NA	3281	Biliary atresia
Ctr9	S	F	73	NA	NA	1273	Familial cholestasis
Ctr10	S	F	96	NA	NA	1101	Sclerosing cholangitis
Ctr11	S	M	49	NA	NA	842	Urea cycle disorder
Ctr12	S	M	12	NA	NA	6734	Biliary atresia
Ctr13	S	F	13	NA	NA	7307	Biliary atresia
Ctr14	S	F	18	NA	NA	NA	Healthy donors
Ctr15	S	M	13	NA	NA	NA	Healthy donors
Ctr16	S	M	25	NA	NA	NA	Healthy donors
Ctr17	S	F	22	NA	NA	NA	Healthy donors

S Serum
T Tissue
OLT Orthotopic Liver Transplantation
CT Chemotherapy
* Age at SLR (Surgical Liver Resection)
NA Not applicable
§ Commercial RNA (Clonetech)
† Dead patient
- Not available
° hepatic resection

Table 1. Clinical features of HB patients (Pt, N=14) and Controls (Ctr, N=17) enrolled in the study

RNA from tissue was normalized by using RNU6B as endogenous controls due to its highest abundance and least variability across normal tissues. RNA from serum was normalized with miR-16 due to its expression at high levels in serum and its relatively low variation across large numbers of samples. Ten nanograms of total RNA were reverse-transcribed using the specific looped primer and quantitative real-time reverse transcription PCR (qRT-PCR) was conducted using the standard TaqMan MicroRNA assay protocol. The 20 μL PCR included 1,33 μL of reverse transcription product, 1× TaqMan Universal PCR Master Mix, No AmpErase UNG, 0.2 μmol/L TaqMan probe, 1.5 μmol/L forward primer, and 0.7 μmol/L reverse primer. The reaction was carried out in a 96-well PCR plate at 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Each sample was analyzed in triplicate.

The levels of miR were measured using Ct (threshold cycle). The amount of target, normalized to an endogenous reference and relative to a calibrator, is given by $2^{-\Delta\Delta Ct}$ (comparative Ct method; ABI user's bulletin 'Relative Quantitation of Gene Expression: ABI PRISM 7700 Sequence Detection System: User Bulletin 2: Rev B). Real-time PCR was performed using the 7500 Fast Real-Time PCR System (Applied Biosystems).

Statistical analysis

Each experiment was repeated at least three times independently. All results were expressed as mean (standard deviation), and $p < 0.05$ was used for statistical significance. One-Way ANOVA analysis for independent samples was used to determine statistically significant differences.

Results

Patient characteristics

Clinical and histopathologic characteristics of patients and controls enrolled in our study are presented in Table 1 and in Table 1S (supplementary data).

All patients had a formal diagnosis of HB. The tumours were examined centrally (General Surgery Department and Liver Transplant Center of the AOU Città della Salute e della Scienza di Torino, Italy) and classified as wholly epithelial and well differentiated fetal (N=6), mixed epithelial and mesenchymal (N=3), mixed embryonal and fetal (N=5) (Table1S).

PRETEXT staging was type III in 5 patients, type IV in 6 and type II in 3. All patients underwent a SIOPEL-guided neo-adjuvant chemotherapic treatment (SIOPEL II, N = 1; SIOPEL III, N=6; SIOPEL IV, N=7).

Surgery consisted in liver resection in 3 cases (Pt5, Pt6, Pt8) and liver transplantation in the other 11. Age at transplantation in HB patients ranged from 8 to 63 months (15 to 143 months for the 3 liver resections) and male to female ratio was 1.33:1.

After transplantation, 2 cases were diagnosed with acute rejection (Pt3 and Pt10), which was treated with steroids. Furthermore, one post-transplant lymphoproliferative disease was detected (Pt1) and treated with a reduction of Tacrolimus in association with Rituximab. Finally, one patient developed pneumonia (Pt11) and was treated with antibiotic therapy. Surgical complications involved bile ducts in two patients (Pt12 and Pt9): one was managed and treated with biliary reconstruction, the other was re-transplanted (Pt9). Bowel volvulus was diagnosed in Pt3 who underwent emergency surgery.

Postoperative chemotherapy was administered, according to SIOPEL protocols, to all patients and no recurrence was detected so far [9]. In the follow-up, patients received periodic physical examinations, laboratory investigations which included complete blood count, liver function tests, alpha-fetoprotein level, and ultrasonography.

The mean follow up is now of 1,375 days. All children enrolled in the study are currently alive, except two (Pt2 and Pt4) who died due to complications after transplantation and HB recurrence, respectively. One resected patient was lost at follow-up (Pt6). Pt4 was a liver sample explanted for HB and matched with its non-neoplastic part (Ctr3).

miR-483-3p and miR-483-5p expression in tissues

We previously demonstrated that whole miR profiling identified differentially expressed miR that was commonly deregulated in HB patients [13]. It was found that 33 of 51 filtered miR were differentially expressed: 21 of them resulted to be up-regulated and 12 were down-regulated. Among these miRs we also observed a large heterogeneity in miR-483 expression. When we analyzed the ratio of the two forms (-3p and -5p) we found that the -5p form was higher than the -3p in tumoral tissue compared to non tumoral ones. In order to validate this observation we performed quantitative real-time PCR on surgical HB tissue samples. For this purposes we used probes corresponding to miR-483-3p and miR-483-5p.

Figure 1 shows miR-483-3p/5p ratio on a log scale. MiR-483-3p levels were higher than those observed for miR-483-5p in control Ctr1 and Ctr2 RNAs. Conversely in Ctr 3 (non–neoplastic part of Pt4) and in HB patients (Pt1-Pt7), ratio was inversed. Collectively, the absolute expression of the two forms did not correspond to an increased level of the transcription of a predominant isoform.

miR-483-3p and miR-483-5p expression in serum

To extend our observation, we performed quantitative real-time PCR on serum samples from patients in follow-up after liver transplantation or surgical resection. In particular we analyzed 10 HB serum samples versus 14 controls. As observed in tissues, in all HB patients, -5p form levels were higher than -3p ones when compared to controls; vice versa, in control samples the -3p form was predominant (Figure 2).

In two controls (Ctr5, Ctr11) an inversion in the pattern of the ratios was observed. In order to explain this variation, we evaluated whether chemotherapy or adjunctive therapies could have influenced the results. Ctr 5 was affected by biliary atresia and had received basiliximab and Tacrolimus as induction of immunosuppression, while Ctr11 was affected by Urea cycle disorder and had been induced with basiliximab only. As shown in Table 1S also other controls were treated with the same immunosuppressive drugs and no effect on the overall results was observed. So far, we concluded that for both Ctr 5 and Ctr11 no correlation with molecular analyses was found neither with chemotherapy nor with immunosuppressive treatments.
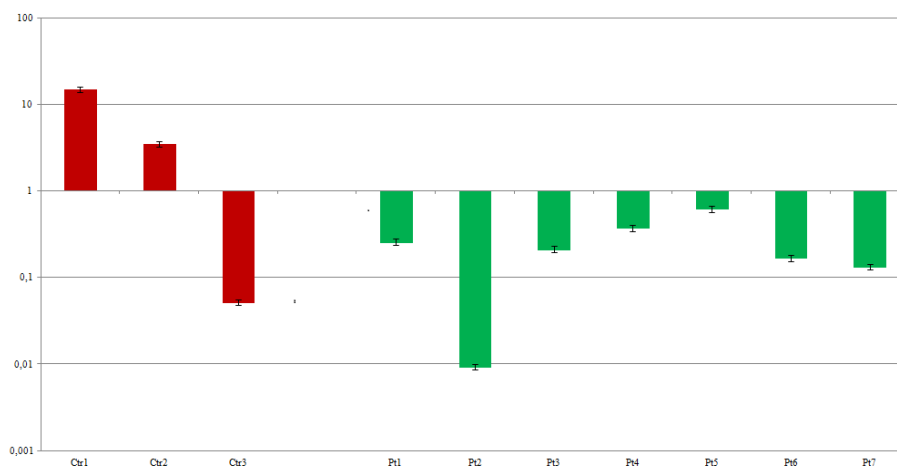

Figure 1: Mir-483-3p/5p ratio (log scale) in tissue samples. MiR-483-3p levels are higher than those observed for miR-483-5p in control Ctr1 and Ctr2 RNAs. Conversely in Ctr 3(non–neoplastic part of Pt4) and in HB patients (Pt1-Pt7) this ratio is inversed.
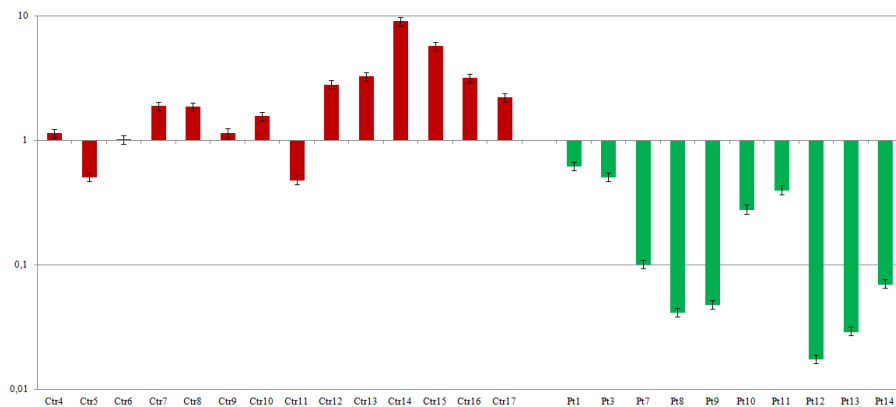
Figure 2: Mir-483-3p/5p ratio (log scale) in serum samples. MiR-483-5p form levels are higher than -3p ones when compared to controls; viceversa, in control samples the miR-483-3p form is predominant. In Ctr5 and Ctr11 an inversion in the pattern of the ratios was observed.

Discussion

Hepatoblastoma is a rare malignant liver tumor affecting infants and many heterogeneous histological tumors subtypes have been described. Although the survival rates have improved dramatically in recent years, there still exists a subset of HB that does not respond to treatment [6]. There are currently no specific molecular biomarkers in use and several studies are aimed at evaluating new potential biomarkers useful in facilitating the early diagnosis.

MiRs comprise a family of highly conserved small noncoding RNAs (-22 nt). As regulators of post-transcriptional gene expression, they play an essential role in a large number of physiological and pathological processes and a number of recent reports have confirmed their usefulness as biomarkers in cancer [38-42]. Furthermore, circulating RNAs in plasma and serum of cancer patients represents an emerging field for noninvasive diagnostic applications. In fact several studies largely focused on distinguishing cancer patients from control subjects using serum or plasma miRs as biomarkers [43,44].

Regarding liver diseases, numerous reports have demonstrated that alterations in the expression of intracellular and extracellular miR correlate with viral-related hepatitis, non-alcoholic steatohepatitis, liver fibrosis and HCC [45,46].

In particular, several miRs have been identified to be implicated in HB tumor onset and progression [22,26]. Among these, miR-483 seems to play an important role in different types of human tumors such as colon, breast, and adult liver cancers, exhibiting different level of expression in about 30% of the cases [30,32,34].

We previously demonstrated that five miRs (miR-214, miR-199a, miR-150, miR-125a, and miR-148a) were helpful in discriminating HB from hepatocellular carcinoma (HCC), thus representing useful markers for the classification of pediatric liver tumors [13].

Data from gene expression profile in our work also highlighted an heterogeneity in the expression of miR-483, being some samples upregulated and some others not. MiR-483 gene is located on genomic chromosome at 11p 15.5 in the second intron of IGF2 and produces two mature forms (miR-483–5p, miR-483–3p). The functions of both forms have been sparsely studied to date and its relevance to carcinogenesis remains unclear.

In the classic model of miR biogenesis, the duplex of mature miRNAs is produced by Dicer processing of the hairpin precursor. Then, a following strand selection step determines which mature miR will act as guide for the mature miR-mediated silencing complex (miRISC). The strand selection process is finely regulated and may vary among tissues and between normal and malignant cells.

Recent studies have been introduced innovative concepts and unraveled that both sister mature miR may be accumulated in some tissues and cell types. In this scenario, the strand selection might not be deterministic but tissue-specific, so that and a given strand could be the guide strand in a specific cell type and passenger in another one [47]. In this respect different biological contexts most likely share complex mechanisms of strand selection regulation, leading either to alternative expression of a single mature form or to concurrent expression of both mature sister miR [48].

MiR-483-3p has been found to be upregulated in 100% of Wilms' tumors and in 33% of HCCs via IGF2 and beta catenin pathways [27]. IGF2 is frequently overexpressed in pediatric cancers.

Recently, it has been shown that the different expression of miR-483 contribute to the development of HCC from liver fibrosis, which was previously suggested to be a continuous process. These observations lead to the hypothesis that that different cell types should be evaluated individually in the development of liver fibrosis to HCC. All these data suggest that a more complex molecular mechanism creates is involved in these continuous changes [36].

The potential involvement of miR-483-3p in common human neoplasms has been widely studied in recent years. In particular, the overexpression of at least two functional elements from the IGF2 locus, the IGF2 protein and miR-483-3p, was considered to be essential in promoting tumorigenesis.

In our study, we evaluated the expression of both miR-483-3p and miR-483-5p in 14 unrelated confirmed cases of HB patients versus non-HB patients. The first evidence emerging from our analysis was that, on the contrary of what is described in other liver pathologies, the quantitative expression of the two forms did not significantly change along the two groups.

Secondly, by the comparison of the ratio 3p/5p we observed an unbalance between HB and non-HB samples. In fact, while in the non-HB group a positive ratio was observed, in the HB samples the ratio was in favor of the 5p form.

Furthermore we also evaluated the potential influence of concomitant treatments in the expression of miR-483 and no correlations were found in patients undergoing to chemotherapy and/or immunosuppressive regiments.

Finally in non-HB patients treated with standard therapy (i.e. immunosuppressive, antibiotics,etc.) for unrelated HB diseases, as well as healthy donors, no correlations with the treatment were found.

These results suggest that, despite the stage of the tumor and/or unrelated diseases or healthy status, miR-483-3p/5p ratio is not influenced by any treatment.

On the basis of our results we hypothesize that HB patients could present a specific molecular signature correlating miR-483-5p strand selection with HB; this hypothesis is supported by the preliminary observation in the only case available of matched HB vs non-HB sample from the same patient (Figure 1).

Such preference may persist after tumor cure, thus representing a possible molecular marker to support classical diagnosis. As shown in Figure 1 and 2, HB samples from both tissue and serum are characterized by a positive ratio of miR-483-5p/3p, On the contrary the ratio was inverted in the majority of the non-HB samples, including healthy donors and patient undergoing to liver transplantation for pathologies different from HB. MiR-483-3p and -5p signature identified in HB patients may serve as a specific fingerprint for improving the accuracy in the diagnosis of this tumor. Even though at the moment it is not possible correlate the miR-483 expression with a specific HB histological subtype, we believe that the analysis of its two different forms may be useful in the molecular identification of HB patients and in distinguishing HB patients from non-HB ones (e.g. from HCC patients). We also suggest that the serum-based miR expression analysis could serve as novel noninvasive biomarkers in HB detection.

In conclusion, even if the involvement of miR in cancer onset and progression has been well described, a possible different strand selection between normal and cancer tissues has not been characterized yet. In this study the expression of the two forms of miRNA-483 was considered and particular attention was paid to the expression of the sister strands (-3p and -5p) in patients affected by HB. The two strands have different targets and are involved in different pathways. The alteration of the miR profile in the tumour microenvironment may be one of the strategies adopted by the tumor for its survival. For this reason we believe that further studies are necessary to elucidate the mechanisms that regulate this important biological process.

Acknowledgement

Conflict of Interest

The authors have no potential conflicts of interest to disclose.

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