Downregulation of sorting nexin 10 is associated with overexpression of miR-30d during liver cancer progression in rats

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Abstract
As of 2012, liver cancer was the second leading cause of death worldwide, and hepatocellular carcinoma is the most common primary cancer of the liver. The identification of molecules that might be molecular markers or therapeutic targets is urgently needed to improve clinical management. Based on a microarray analysis performed in our laboratory, we selected six genes—namely, ANXA2, DYNLT1, PFKP, PLA2G7, KRT19, and SNX10—as candidates for validation as tumor markers of liver cancer in a rat model. Their patterns of overexpression in preneoplastic lesions and established tumors at 10 different time points between 24h and 18 months were analyzed to identify putative tumor markers for further studies. We validated the microarray results by quantitative reverse transcription polymerase chain reaction, which revealed high transcriptional expression for five of the genes, consistent with their high protein expression during cancer progression reported in the literature. However, studies of the association of sorting nexin 10 with different types of cancer are limited, prompting further study. The characterization of sorting nexin 10 in preneoplastic lesions and established tumors revealed messenger RNA overexpression and a simultaneous decrease in sorting nexin 10 protein expression. A group of microRNAs related to sorting nexin 10 messenger RNA were selected based on a data analysis conducted using miRDB and microrna.org. An analysis of the expression of these microRNAs revealed an increase in the transcription of microRNA-30d whenever the sorting nexin 10 protein was downregulated. These results suggest that sorting nexin 10 is a potential liver cancer marker exhibiting characteristics of a putative suppressor protein that is likely regulated by microRNA-30d.

Keywords
Sorting nexin 10, microRNA, hepatocellular carcinoma, tumor suppressor

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Introduction
In 2012, 14.1 million new cancer diagnoses and 8.2 million cancer-related deaths were registered worldwide. Specifically, 782,000 new cases of liver cancer were reported, and 745,000 deaths due to liver cancer were recorded in the same year, making liver cancer the second most common cause of cancer-related deaths. Hepatocellular carcinoma (HCC) is the most common primary cancer of the liver in men worldwide.¹ In the United States, from 2009 to 2012, a 3.1% decrease in cancer was

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observed among men due to a decrease in the diagnosis of prostate cancer. Since 1991, the mortality rate has decreased by 23%, corresponding to the prevention of more than 1.7 million deaths through 2012. Despite this progress, the mortality rate is increasing for cancers of the liver, pancreas, and uterus. In 2012, an estimated 35,236 new cases of liver cancer were diagnosed, and 27,170 deaths were recorded. Despite general improvements in the clinical management of cancer, the incidence of liver cancer has increased in 21 states in the United States. These observations are a clear indication of the urgent need to identify new macromolecular markers of HCC for early diagnosis and to serve as new therapeutic targets for the successful clinical management of this disease.

Hepatocarcinogenesis is a complex process associated with the accumulation of genetic and epigenetic changes that occur in three stages: initiation, promotion, and progression. These three stages have been identified in different experimental animal models to complement cancer studies in humans. We characterized cancer marker genes in a modified resistant hepatocyte model of chemical hepatocarcinogenesis during cancer progression at 10 time points, ranging from 24 h to 18 months. A previous microarray analysis identified genes that were overexpressed in preneoplastic lesions and well-established HCCs, despite the overexpression of SNX10 mRNA. These findings led us to examine the potential regulation of SNX10 by microRNAs (miRNAs), which could be the cause of the discrepancy between the expression of the SNX10 protein and that of its mRNA. Our findings reveal a possible connection between the suppression of SNX10 mRNA translation and the expression of an miRNA, and thereby establish a basis for further studies on SNX10 in liver cancer progression.

**Materials and methods**

**Reagents**

The following reagents were used: diethylnitrosamine (Sigma-Aldrich, St. Louis, MO, USA), 2-acetylaminofluorene (2-AAF; Sigma-Aldrich, St. Louis, MO, USA), 2-methyl butane (J.T. BAKER, USA), gamma-glutamyl transpeptidase enzyme (GGT; SIGMA, Germany), l-glutamic acid γ-(4-methoxy-β-naphthylamide) (GMMA; SIGMA, Germany), dimethyl sulfoxide (DMSO), glycyglycine, Fast Blue (Sigma-Aldrich, St. Louis, MO, USA), TriPure isolation reagent (Roche, REF 11667165001 Cat. No. 18064-014, Mannheim, Germany), RT kit with 5x reverse transcription (RT) buffer, 0.1 M dithiothreitol (DTT), 10mM deoxynucleotide triphosphates (dNTPs), SuperScript II Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA), TaqMan Universal PCR Master Mix (Applied Biosystems, Branchburg, NJ, USA), Bio-Rad DC Protein Assay Reagents (Bio-Rad, Hercules, CA, USA), Cat. Nos. 500-0113 (Reagent A), 500-0114 (Reagent B), and 500-0115 (Reagent S), USA), Luminol (Santa Cruz Biotechnology, Inc., CA, USA), Moloney murine leukemia virus (MMLV) RT enzyme (Invitrogen), random oligo primers (Invitrogen), SYBR Green qPCR Kit (Thermo Scientific, Waltham, MA, USA), polyvinylidene difluoride (PVDF) membranes (Millipore Corporation, Billerica, MA, USA), mirVana miRNA Isolation kit (Ambion, Part No. AM 1561, USA), anti-SNX10 antibody (Santa Cruz Biotechnology, Inc., Cat. No. Sc-104657, CA, USA), and secondary antibody (Invitrogen).

**Animals**

All experiments were performed in accordance with and under approval from the Internal Committee for the Care
and Use of Laboratory Animals of the Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN, Ciudad de México, México; Protocol No. 0001-02). Male Fischer 344 rats weighing between 180 and 200 g were obtained from the Animal Production Unit of the Experimental Laboratory (UPEAL Cinvestav, Mexico City, Mexico). The animals had free access to food (PMI Foods, Inc., Lab Diet, Richmond, IN, USA) and water and were maintained under controlled conditions with 12-h light/12-h dark cycles, 50% relative humidity, and a temperature of 21°C.

**Experimental protocol**

At the beginning of the experiment, the animals were intraperitoneally administered a dose of 200 mg/kg DEN, followed by i.g. doses of 20 mg/kg 2-AAF on days 7, 8, and 9. A 75% partial hepatectomy was performed on day 10. Four animals were sacrificed on days 1, 7, 11, 16, and 30 and at 5, 9, 12, and 18 months (Figure 1). Their livers were excised, washed in physiological saline solution, frozen in 2-methyl butane with liquid nitrogen, and stored at −80°C.

### Staining for the detection of GGT activity

A histological analysis of preneoplastic and neoplastic lesions was performed using a histochemical assay for the enzymatic activity of GGT. Sections of liver tissue with a thickness of 20 µm were mounted on gelatinized slides and treated with 2.5 mg of GMMA, 50 mL of DMSO, deionized water, 25 mM Tris (pH 7.4), 0.5 mg of glycylglycine, and 0.5 mg of Fast Blue. Digital images were obtained, and the GGT-positive regions in preneoplastic and neoplastic lesions of liver tissue from the animals sacrificed at 1, 5, 9, 12, and 18 months were detected to identify material for subsequent extraction of RNA and total protein (Figure 2).

### RNA extraction

For the extraction of RNA and proteins, 1 mL of reagent TriPure was added to 60 mg of liver tissue, and the mixture...
was homogenized and centrifuged for phase separation. The aqueous phase containing the RNA in solution and the total precipitated protein was processed using the TriPure reagent kit (Roche, Mannheim, Germany) following the instructions included in the manual.

To RNA contained in the aqueous phase, 0.5 mL of isopropanol 100% was added per milliliter of TriPure reagent used in the initial homogenization, and the mixture was incubated at room temperature for 10 min and centrifuged at 12,000g for 10 min at 4°C. After the supernatant was removed, the precipitated RNA was washed with 1 mL of 75% ethanol per each milliliter of the TriPure reagent used in the initial homogenization. The sample was then centrifuged at 7500g and 4°C for 5 min, and the pellet was resuspended in RNAse-free water for storing at −70°C. The RNA was quantified spectrophotometrically at OD260, and the RNA integrity was evaluated through electrophoresis of the RNA samples on 1% agarose gels.

The RNA used for miRNA analysis was extracted from liver tissue using the mirVana kit according to the instructions provided in the manual. The liver samples were lysed in a denaturing solution, which stabilizes RNA and inactivates RNases. The lysate was then extracted once with acid-phenol:chloroform, which removes most of the other cellular components to yield a semi-pure RNA sample. This sample was then further purified with the glass fiber filter, which uses solutions formulated specifically for miRNA retention to avoid the loss of small interfering RNAs.

**Protein extraction**

For protein isolation, the precipitates obtained from the phase separation were treated with 1.5 mL of isopropanol per milliliter of the TriPure reagent that was initially employed during homogenization. The samples were then incubated for 10 min at room temperature and centrifuged at 12,000g and 4°C for 10 min. After the supernatant was discarded, the protein pellet was washed with 2 mL of 0.3M guanidine chloride solution in 95% ethanol per milliliter of TriPure reagent initially employed. After washing, the sample was centrifuged at 7500g and 4°C for 5 min, the supernatant was removed, and 2 mL of 100% ethanol was added to the pellet. The mixture was then stirred, incubated for 20 min at room temperature, and centrifuged at 7500g and 4°C for 5 min. The ethanol was removed, and the protein pellet was allowed to dry for 5–10 min. Afterward, 200 µL of 1% sodium dodecyl sulfate (SDS) was added, and the pellet was resuspended and centrifuged at 10,000g and 4°C for 10 min. The pellet with the proteins was transferred to a new tube for storage at 20°C.

**RT**

The complementary DNA (cDNA) for the qPCR assay was prepared from 2µg of total RNA using an RT kit (Invitrogen). Each reaction mixture contained 4µL of 5× RT buffer, 1µL of 0.1M DTT, 1µL of 10mM dNTPs, and 0.5µL of reverse transcriptase. The reaction was performed in a thermocycler (Applied Biosystems) using a protocol consisting of cycles of 16°C for 30 min, 42°C for 30 min, and 85°C for 5 min.

**qRT-PCR**

qRT-PCR was performed using TaqMan Gene Expression Assays with a One-Step Real-Time PCR System (Applied Biosystems). The PCR reaction mixture contained 1µL of cDNA, 7.5µL of TaqMan Universal PCR Master Mix, 0.4µL of the TaqMan probe, 1µL of tempered cDNA, and 6.1µL of RNase-free water. The following cycling protocol was used: 1 cycle of 50°C for 2 min, 1 cycle of 95°C for 10 min, and 40 cycles of 95°C for 15s and 60°C for 1 min. The 18S ribosomal RNA (18S rRNA; Rn18s) gene was used as an internal control. The results from four independent replicates were employed for statistical calculations. The relative gene expression was calculated using the 2−ΔΔCT method. The sequences for the primers and probes used for ANXA2, DYNTL1, PFKP, PLA2G7, KRT19, SNX10, and 18S rRNA were obtained from Applied Biosystems with the following identification numbers: Rn00571516_m1, Rn01526256_m1, Rn01487769_m1, Rn757268_m1, Rn01496867_m1, Rn01763032_m1, and Rn01428915_g1, respectively.

**Western blotting**

Protein extracts were obtained following the TriPure protocol. Total liver homogenates were collected from the early time points—namely, days 0, 1, 7, 11, and 16—and liver fragments from GGT-positive regions and adjacent areas were collected from the later time points—namely, 1, 5, 9, 12, and 18 months. The protein concentrations for each sample were determined using the Bio-Rad DC Protein Assay kit. Then, 60µg of protein was prepared in loading buffer containing SDS, glycerol, 1M Tris–HCl (pH 6.8), β-mercaptoethanol, and bromophenol blue. The samples were subsequently denatured by heating for 10 min and loaded into a 15% SDS-polyacrylamide gel electrophoresis (PAGE) gel. After electrophoresis, the proteins were transferred to a PVDF membrane that had been blocked with blocking solution (5% skimmed milk and 1% albumin dissolved in 1× phosphate-buffered saline (PBS; NaCl, KCl, KH2PO4, and Na2HPO4)). The membrane was then incubated overnight at 4°C with a primary anti-SNX10 antibody (Santa Cruz Biotechnology) and then for 1 h at room temperature with the appropriate secondary antibody conjugated to horseradish peroxidase (HRP; Invitrogen). The proteins were detected using luminol. All tests were performed with four independent replicates.
**qRT-PCR of miRNAs**

Two micrograms of each miRNA sample was converted into cDNA using the MMLV RT enzyme at 42°C with an oligo-dT primer. The Maximum SYBR Green qPCR kit was used for qPCR analysis. Each 15-µL reaction contained 100 ng of cDNA, 7.5 µL of the 2× SYBR Green reaction mixture, 0.5 µL of specific oligonucleotides (10 pmol/µL), and 6.5 µL of water. The reaction conditions were as follows: 95°C for 15 min followed by 40 cycles of 95°C for 45 s and 60°C for 1 min. The analysis does not distinguish the expression of mature miRNAs from that of pri- or pre-miRNAs.

The following oligonucleotides were designed using Oligo Analyzer software and used for the amplification of miR-30b5p, miR-30c, miR-30d, miR-30e, miR-30ade, and miR-U6.

**Statistical analysis of data**

A data analysis for the assessment of differential gene expression was performed using one-way analysis of variance followed by Tukey’s post hoc analysis. Genes were considered to be differentially expressed when they displayed significant changes, as indicated by a p value less than 0.05. The transcription of miRNAs was quantified using the comparative Ct method.

**Results**

**Validation of selected genes via qRT-PCR**

As shown in Table 1, a group of overexpressed genes exhibiting very small variations in expression in the age-control groups at 9 and 18 months was identified. A microarray analysis of the transcriptomic expression profiles of ANXA2, DYNLT1, PFKP, PLA2G7, KRT19, and SNX10 indicated robust overexpression in preneoplastic lesions at 30 days and in tumors at 9, 12, and 18 months (Table 1). In contrast, their expression was lower in the adjacent areas. The control values for the non-treated rats at 9 and 18 months showed similar gene expression to the control values at time 0 (Figure 3).

The values obtained for the six selected genes by real-time qRT-PCR were higher than those obtained from the microarray analysis. For example, the expression of PLA2G7 in the 12 mT group presented a 50.77-fold increase according to the microarray analysis and a 729.22-fold increase according to qRT-PCR. However, the observed changes were always in the same direction. The maximum fold-changes in the expression of the six genes obtained through the RT-PCR assays were as follows: ANXA2, 14.2 at 9 mT; DYNLT1, 39.56 at 12 mT; PFKP, 6.5 at 18 mT; KRT19, 16.12 at 18 mT; PLA2G7, 729.22 at 12 mT; and SNX10, 55.79 at 12 mT (Figure 3).

Among the six validated genes, SNX10 and PLA2G7 exhibited the highest fold-changes in gene expression, presenting values greater than 15 and up to 55.79 in well-established preneoplastic lesions and neoplastic lesions (at 30 days, 9 mT, 12 mT, and 18 mT). With the exception of SNX10, some information is available in the literature regarding the participation of the other five genes in cancer progression.7–11 The limited information related to the SNX10 protein and its relationship with cancer, together with the notable increase in SNX10 gene expression, prompted us to analyze the SNX10 protein via western blotting.

**SNX10 protein analysis**

Unexpectedly, although the SNX10 transcript showed 24.36-, 39.21-, and 25.94-fold upregulation according to the qRT-PCR assay at 30 days, 9 mT, and 18 mT, respectively, the protein level of SNX10 was downregulated at these points, with values of −0.05, 0.13, and −0.93, respectively. At 12 mT, the protein showed a slight increase of 7.64-fold, whereas the fold-change obtained by qRT-PCR was 55.79. Representative western blot assays are presented (Figure 4(a) and (b)), and a graph showing the quantification of the SNX10 transcript at the time points at which it was overexpressed is also shown (30 dN, 9 mT, 12 mT, and 18 mT; Figure 5).

**Analysis of miRNA-30b5p expression**

Due to suppression of the SNX10 protein in the rat liver at the time points at which SNX10 gene expression was high,

| Gene name | 11 days | 30-day nodular tissue | 9 months control | 9 months tumor | 12 months tumor | 18 months control | 18 months tumor |
|-----------|---------|----------------------|------------------|--------------|----------------|------------------|----------------|
| ANXA2     | 9.92    | 10.04                | −1.15            | 11.0         | 7.84           | 1.04             | 17.17          |
| DYNLT1    | 4.67    | 2.37                 | −1.69            | 6.00         | 3.26           | −1.05            | 3.26           |
| PFKP      | 4.13    | 2.22                 | −1.08            | 3.23         | 3.82           | −1.06            | 4.15           |
| PLA2G7    | 8.83    | 11.58                | −1.06            | 57.12        | 50.77          | 1.19             | 129.96         |
| KRT19     | 6.16    | 6.59                 | −1.50            | 13.93        | 12.0           | −1.06            | 12.50          |
| SNX10     | 4.78    | 4.40                 | −1.29            | 10.83        | 5.93           | −1.30            | 11.10          |
Figure 3. qRT-PCR validation of overexpressed genes. Graphs show the average expression of three replicates (±SD) of ANXA2, DYNLT1, PFKP, PLA2G7, KRT19, and SNX10 in non-treated rats (white columns), preneoplastic lesions (gray columns), and established HCC tumors (black columns). The 18S rRNA gene was used as an internal control. Asterisks indicate a significant difference (p < 0.05) in comparison with non-treated rats at the beginning of the experiment.

Figure 4. Analysis of SNX10 expression by western blotting. Densitometric analysis of the western blot. Samples were analyzed as quadruplicates ± SD, and actin was used as loading control. Asterisks indicate a significant difference (p < 0.05).
we performed an in silico analysis to identify a putative miRNA regulator of SNX10 expression using the miRDB (mirdb.org/miRDB) and microrna.org (www.microrna.org/microrna/home.do) databases. The results of this in silico analysis are shown in a Venn diagram (Figure 6) and in Supplementary Table 1, and these data show that miR-30b5p was identified in Rattus norvegicus, specifically rno-miR-30b5p in miRDB (score of 76) and microrna.org, and in Homo sapiens, specifically hsa-miR-30b5p in miRDB (score of 77). Other putative regulatory miRNAs for SNX10 mRNA identified in the analysis of the databases are miR-30c (score of 74), miR-30d (score of 75), miR-30e (score of 75), and miR30-ade (score of 70). In addition, miR-384-5p (score of 73) was identified in R. norvegicus. Among the six miRNAs shown in the Venn diagram presenting high miRNA complementarity with the 3′ untranslated region (UTR) region of the SNX10 mRNA, miR-30b5p exhibited the highest complementarity. To analyze these different miRNAs, we used oligonucleotides designed using Oligo Analyzer software. We determined the expression levels of this miRNA in hepatocarcinogenesis at 11 days, 30 dN, 9 mT, 12 mT, and 18 mT and in non-treated control rats at 0, 9, and 18 months. The obtained expression values were not different from those of the controls, and miR30b-5p was therefore discarded as an miRNA that is potentially responsible for repression of the SNX10 protein (Figure 7).

Expression of the miRNA-30 family (c, d, e, and ade)

The in silico analysis identified miRNA-30c, miRNA-d, miRNA-e, and miRNA-ade as the miRNAs with the next highest complementarity to the 3′UTR region of SNX10 mRNA. Therefore, we quantified their expression in tumors at 9 months. Our results showed that miRNA-30d was the most overexpressed, presenting up to threefold overexpression in comparison with that in the untreated control at 9 months (Figure 8).

miRNA-30d expression analysis

Within the miRNA-30 family, miRNA-30d exhibited the highest expression. Therefore, we proceeded to determine its expression during the progression of carcinogenesis. The expression of miRNA-30d at 30 dN, 9 mT, 12 mT, and 18 mT, when the SNX10 protein was repressed, showed fold-changes of 7, 2, 10, and 2, respectively. Therefore, the correlation of high miRNA-30d expression with the suppression of SNX10 protein...
Discussion

As a follow-up study of possible candidate early cancer markers identified in our previous microarray study, we selected a group of genes that were overexpressed in cancer and showed very small variations in the age-control group at 9 and 18 months. We performed a gene expression analysis of the following six selected genes: ANXA2, DYNLT1, PFKP, KRT19, PLA2G7, and SNX10. These genes were overexpressed in both preneoplastic and neoplastic lesions compared with the respective adjacent tissues and with liver tissues from non-treated rats. In particular, we focused on SNX10 and further analyzed its expression during cancer development.

SNX10 is a member of the SNX family. The human genome encodes 49 proteins containing the representative phox homology domain (PX) domain, but little is known about their function. The PX domain is responsible for the regulation of the endocytic pathways and secretory pathways as well as remodeling of the membrane for fusion to different organelles. SNX10 is associated with osteopetrosis disease, and SNX10 overexpression can induce the formation of giant vacuoles associated with disruption of vesicles derived from the Golgi to form endosomes.

A gene expression analysis of the six selected genes via qRT-PCR indicated a common pattern of overexpression in preneoplastic lesions from 30-day nodular tissue (30 dN) and neoplastic lesions at 9 months (9 mT), 12 months (12 mT), and 18 months in tumor tissues (18 mT). This pattern is indicative of elevated transcriptional levels and synthesis of proteins participating in cancer progression. This pattern was detected for all of the genes analyzed with the exception of SNX10, which showed an inverse correlation between protein and gene expression.

According to these observations, we hypothesized that SNX10 acts as a suppressor protein and is very likely regulated by miR-30d. miR-30d is one of the family members of the 30S miRNAs, and this family regulates a wide range of physiological processes in normal and cancerous tissues, such as development, metastases, apoptosis, senescence, proliferation, and differentiation. It was recently suggested that miR-30d is a new oncogene involved in tumor development that serves as a potential biomarker or therapeutic target for the treatment of human cancers.

A previous study showed that an allele-specific imbalance of two SNX10 gene variants is related to colon cancer. Thus, the SNX10 gene is differentially expressed in esophageal and colon cancers and is a potential cancer marker. However, this study did not establish whether the SNX10 protein is under- or overexpressed. Further supporting a relationship between the SNX family and cancer progression, another member of this protein family, SNX1, has been proposed as a tumor suppressor.

In support of our suggestion, five other suppressor proteins—namely, SOCS1 in prostate cancer, GNAI2 in liver cancer, GALNT1 and GALNT7 in melanoma cells, ATG5 in autophagy, and CASP3 in ovarian and breast cancers—appear to be regulated by miR-30d in a similar manner as SNX10. The sites of miRNA-30d and mRNA complementarity in GNAI2, SOCS1, GALNT1, and GALNT7 are the same as that in SNX10 (Figure 10). Further supporting the participation of miRNA-30d in cancer progression, in
vitro studies of GNAI2 function have demonstrated that miRNA-30d promotes cell migration, invasion, and metastasis.\textsuperscript{21} Our findings for SNX10 and a report of GNAI2 and SOCS1, and GALNT1 and GALNT7 note a relationship between miR-30d overexpression and suppression of the expression of the respective proteins.

The lines of evidence showing the relevance of miR-30d as an oncomir include the results of a study that found that the oncogene (miR-30d) frequently presents 30\% amplification in a group of 1283 human epithelial tumors. In addition, this oncogene is significantly amplified in eight of the nine types of human cancers studied, namely, bladder, breast, colon, lung, melanoma, neuroblastoma, ovarian, pancreatic, and sarcoma cancers. These findings suggest that miR-30d is a potential oncogene.\textsuperscript{23}

Other genes predicted as targets of the oncogene miR-30d are the following: autophagy-related 5 (ATG5) and caspase 3 (CASP3). The \textit{ATG5} gene is known as a target of miRNA-30d because the binding site for this miRNA has been found in the 3′UTR region of the mRNA. Autophagy plays an important role in the initiation and progression of epithelial tumors through what has been considered a tumor-suppressive function because disruption of genes such as ATG5 leads to tumor initiation. In addition, miR-30d acts as an oncogene by binding and regulating \textit{ATG5} directly.\textsuperscript{18,24} Another example is CASP3, a core protein of cell apoptosis, and results obtained in cell lines of breast cancer and ovarian cancer show that miR-30d regulates cell proliferation, senescence, and apoptosis, suggesting that miR-30d serves as an oncogene in human cancer.\textsuperscript{23} However, two other studies of renal and prostate cancer have suggested that miRNA-30d acts as a suppressor.\textsuperscript{25,26} Unfortunately, there is no clear explanation to resolve this discrepancy.

Based on the overexpression of \textit{SNX10} observed at the transcriptional level in premalignant lesions and in established tumors and the downregulation of its protein when miRNA-30d is overexpressed, we propose that SNX10 is translationally regulated by miRNA-30d. In summary, we present the hypothesis that the binomial miRNA 30d-SNX10 acts as a potential tumor oncogene suppressor by behaving as a dual tumor marker and a dual therapeutic target in liver cancer.

\textbf{Declaration of conflicting interests}

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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