Predictive markers of Sorafenib sensitivity of HCC

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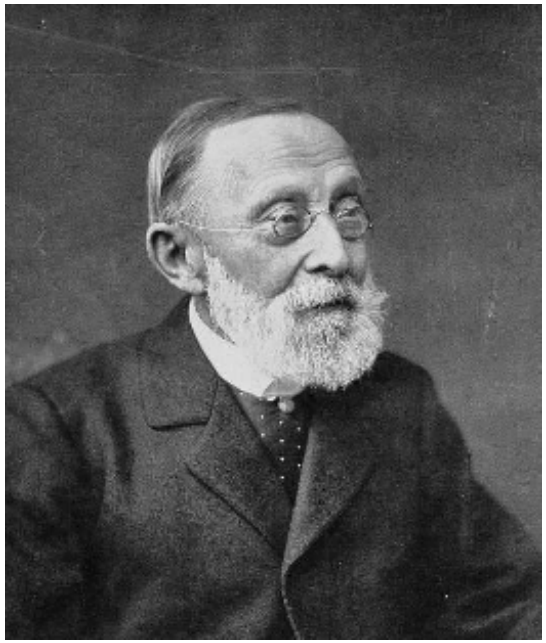
November 06.
2015

Morgagni,1761



Virchow,1858

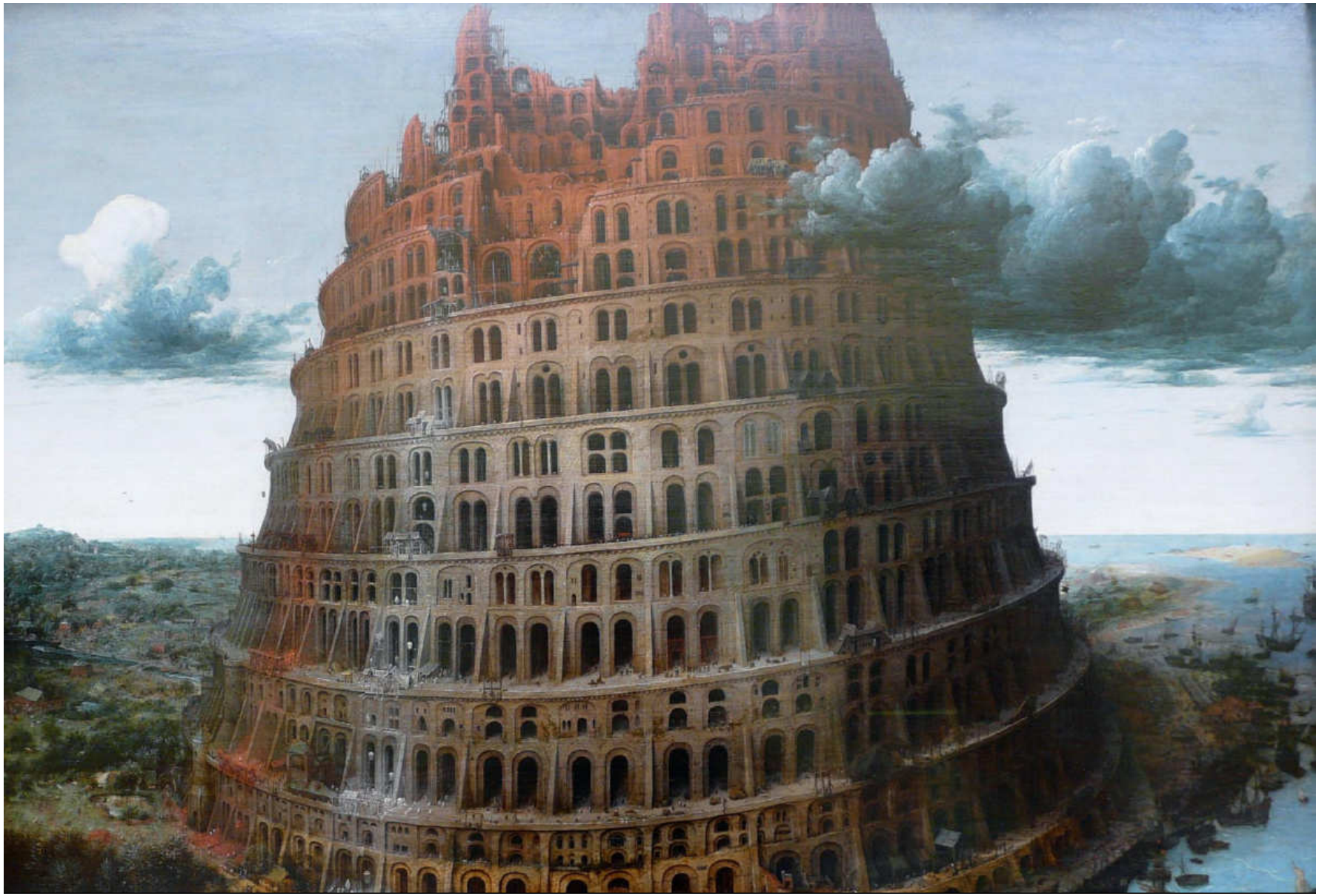
Watson & Creek, 1953

GI Diseases erkezete

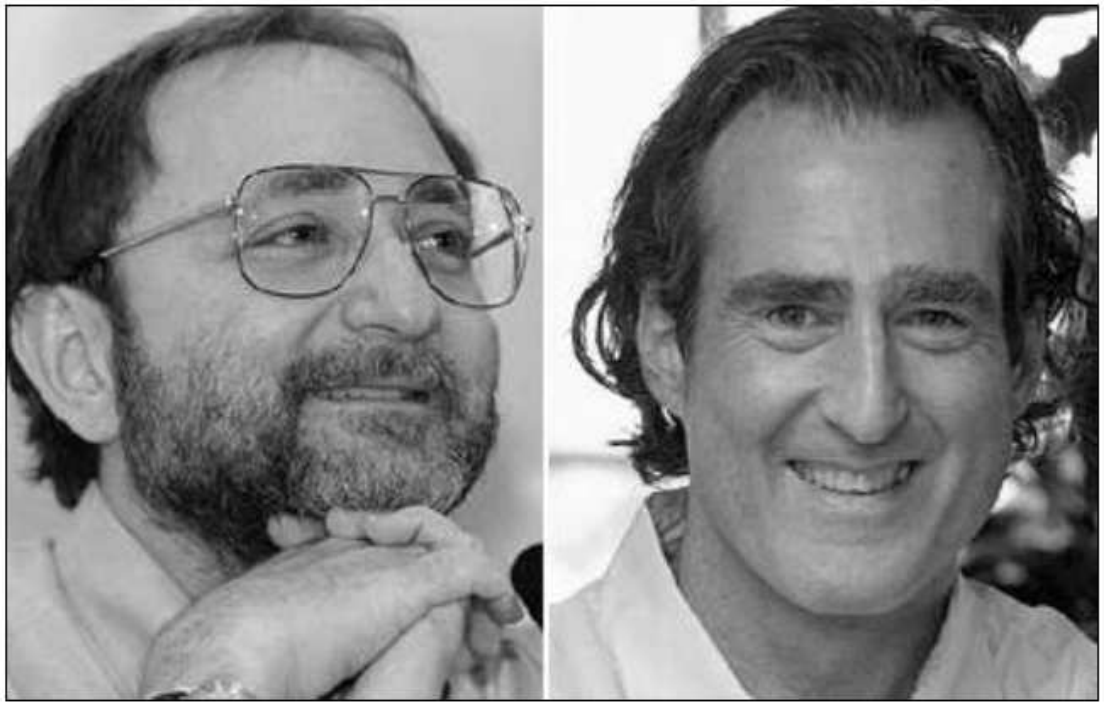
„Zellularpathologie”

DNA structure

World of non-coding RNAs



World of interference . . .



Andrew Z. Fire Nobel prize 2006 Craig C. Mello

...reported a potent gene silencing effect after injecting **double stranded RNA** into *C. elegans*...

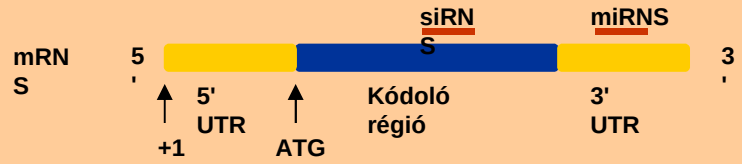
nature Fire A, et al - Nature 1998

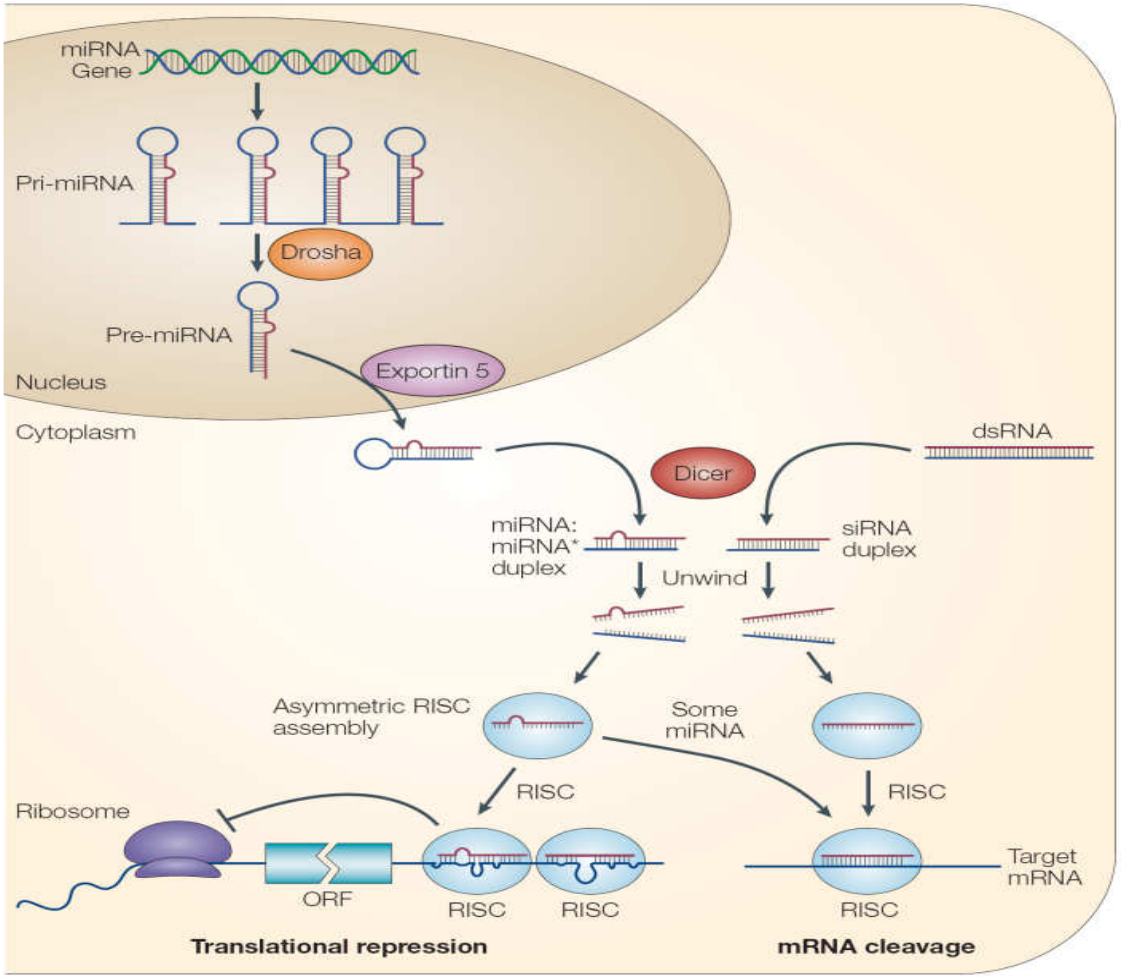
RNS silencing – RNA interference

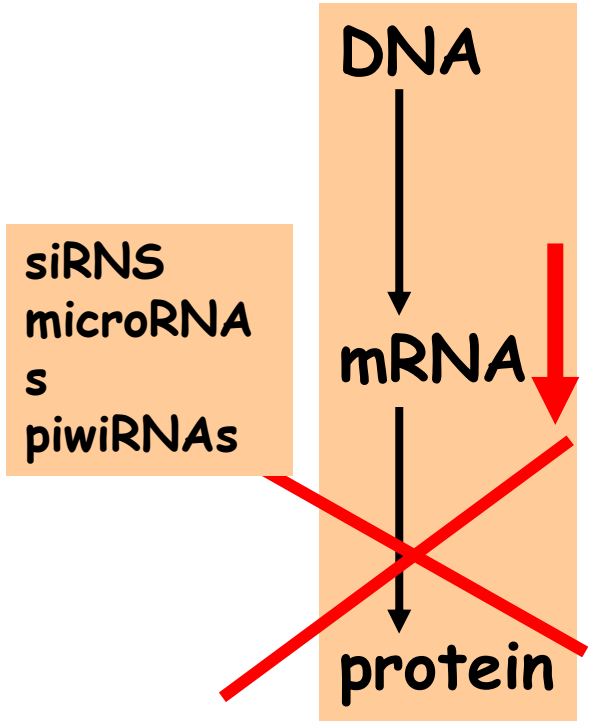
PATHOBIOLOGY – EXPRESSION PATTERN

DIAGNOSTICS

THERAPY

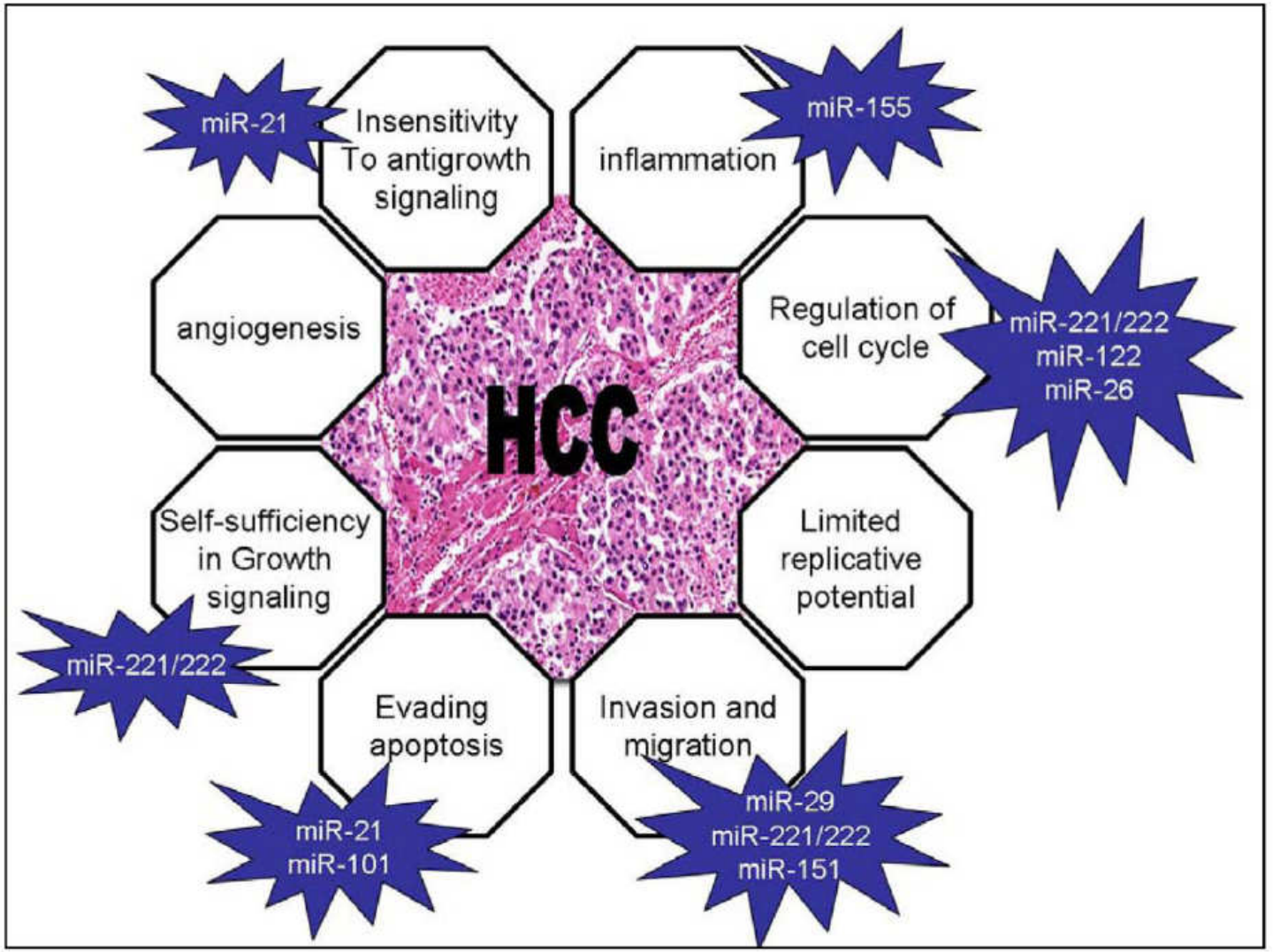






siRNS
microRNAs
piwiRNAs

- 20-22 bp RNA
- Negative regulation
- Posttranscription level
- ~ 2000 mikroRNAs
- Normal cell function
- pathologic conditions characteristic miR profile



Braconi C.et al., Semin. Oncol., 2014
Cortez et al., Nat Rev Clin Oncol, 2011
Braconi C.et al., Semin. Oncol., 2011

Referen				
Autho				
Murakar				25
Pineau				
Gramant				a,
Li-Fu				1
Jiang				0,
Li				c,
Su				1, 23,
Wang				
Connoll			miR-15a, miR-92	let-7a, miR-122

Braconi C.et al., Semin. Oncol., 2014
Cortez et al., Nat Rev Clin Oncol, 2011
Braconi C.et al., Semin. Oncol., 2011

Proved or assumed biological effect of the investigated miRNAs

Oncogenic miRNAs

miR-17-5p	Proliferation
miR-18a	Proliferation, migration (member of the oncogenic miR-17-92 cluster)
miR-21	Proliferation, migration
miR-34a	Inhibits p53
miR-210	Proliferation, antiapoptotic
miR-221	Proliferation, invasion, antiapoptotic
miR-222	Proliferation, invasion, antiapoptotic
miR-224	Proliferation, migration

Tumor suppressor miRNAs

miR-195	Proapoptotic
miR-214	Inhibits invasion, affects β-catenin pathway
miR-223	Inhibits proliferation

Liver characteristic miRNA

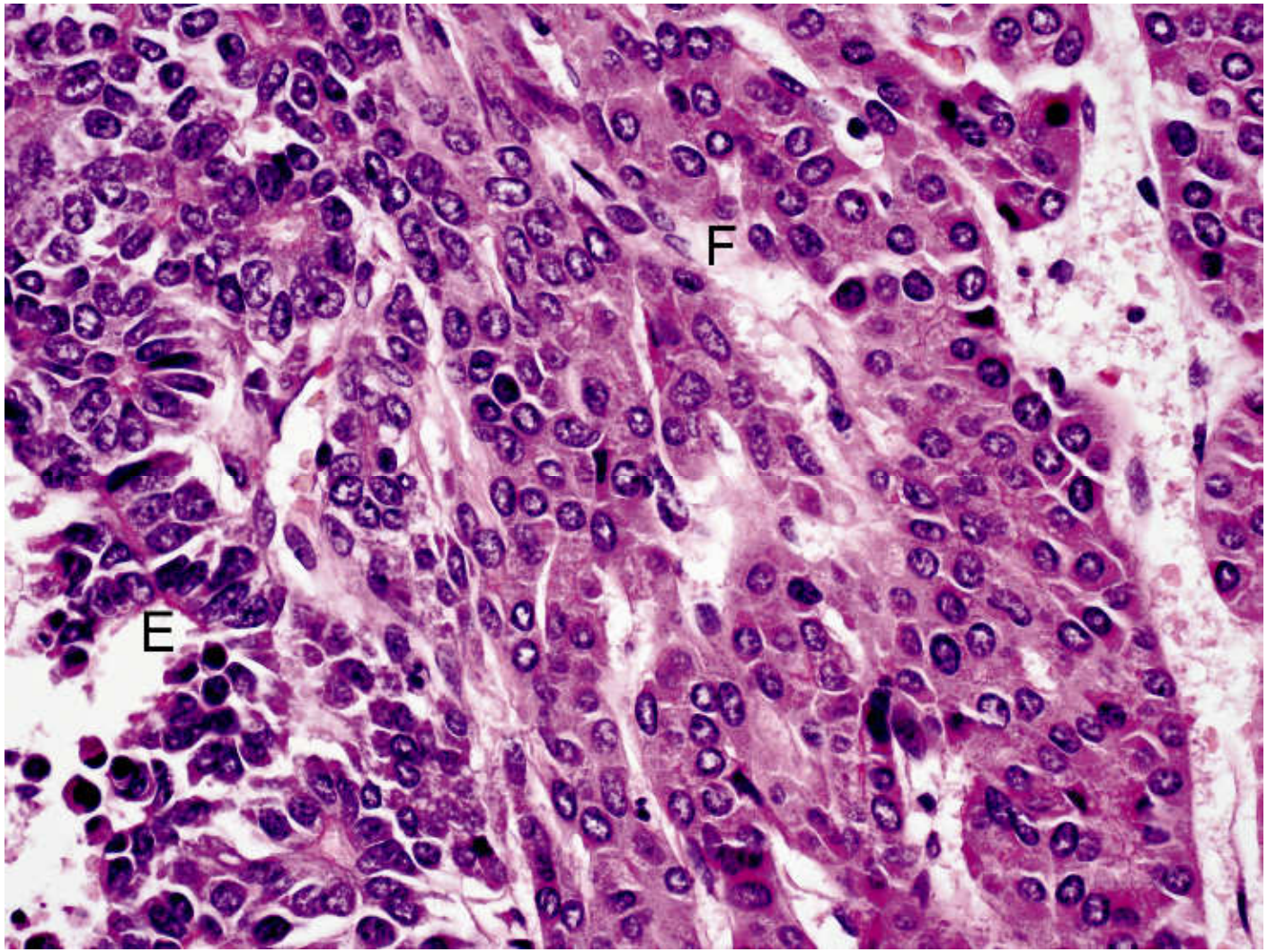
miR-122	Regulates fat metabolism, necessary to normal hepatocyte function Decreased cellular levels contribute to fibrosis, steatohepatitis and HCC

Reference miR

miR-140

senzitizes for sorafenib *in vitro*[1,2]

Reference
1. Yang et al. 2013.
2. Bai et al. 2009.
3. Murakami et al. 2006.

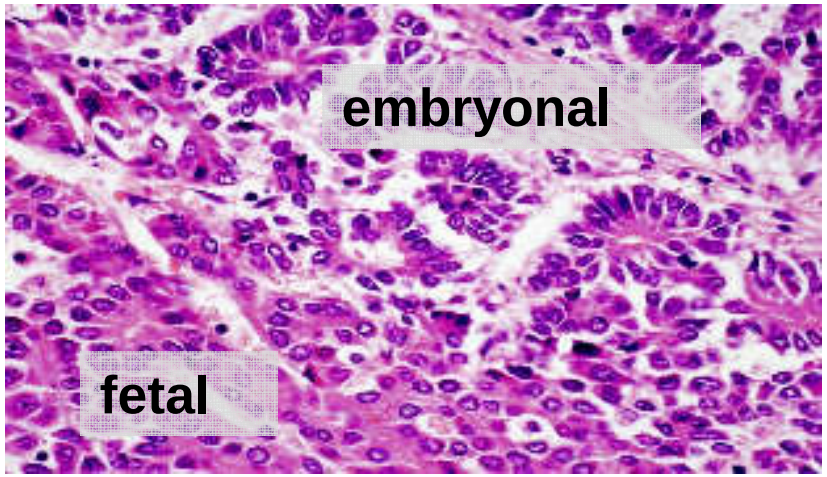


„microRNA expression might predict prognosis of epithelial hepatoblastoma”

Monika Gyugos1, Gábor Lendvai1, István Kenessey1, Krisztina Schlachter1, Judit Halász1, Péter Nagy2, Miklós Garami3, Zsuzsa Jakab3, Zsuzsa Schaff1, András Kiss1#, Virchows Archive, 2014

Clinical data

- 21 human epithelial hepatoblastoma (average: 45.89 months , 11/10 M/F ratio)
- Pure fetal: 12 cases, embrional/fetal: 9 cases. Overall considering more or less than 5 % fetal compartment 16 fetal and 9 embryonal cases were analysed.
- 16 non-tumorous surrounding liver.

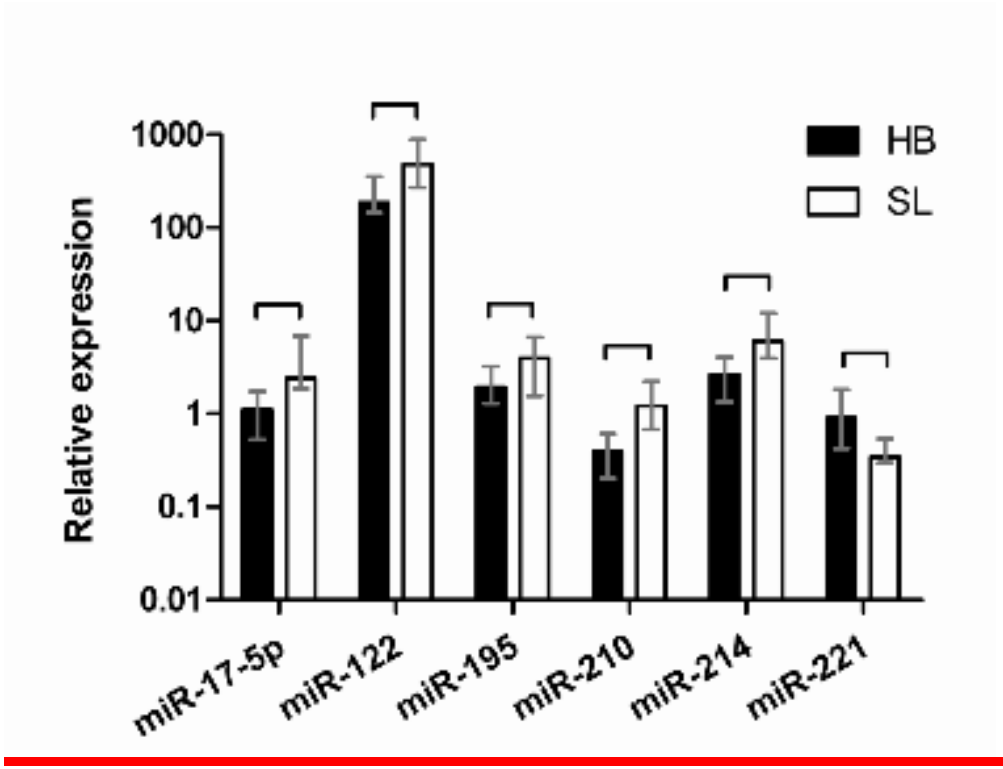


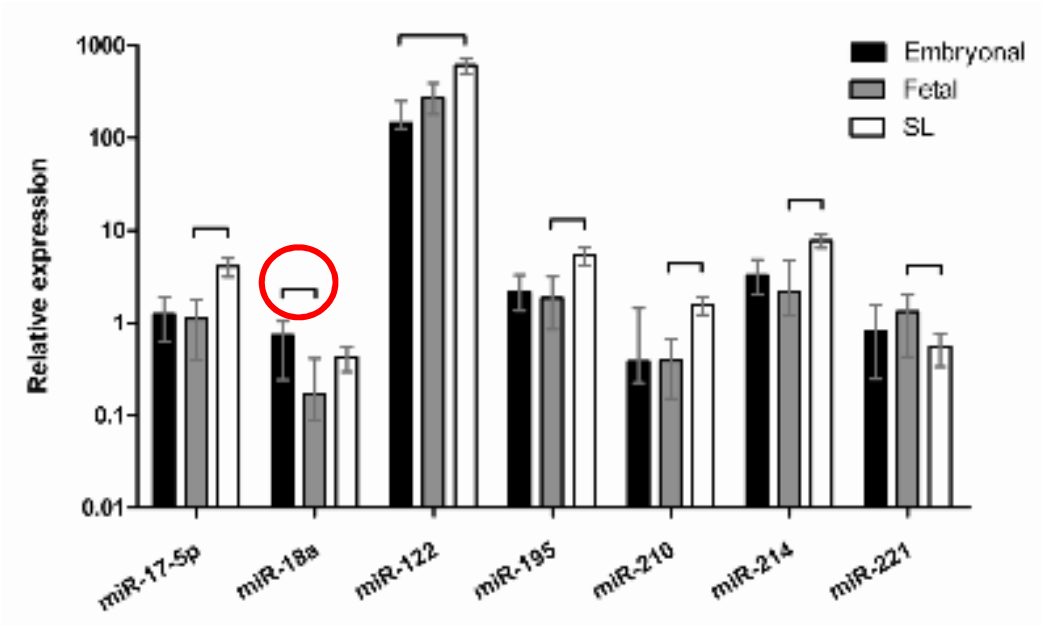
microRNA expression in HB

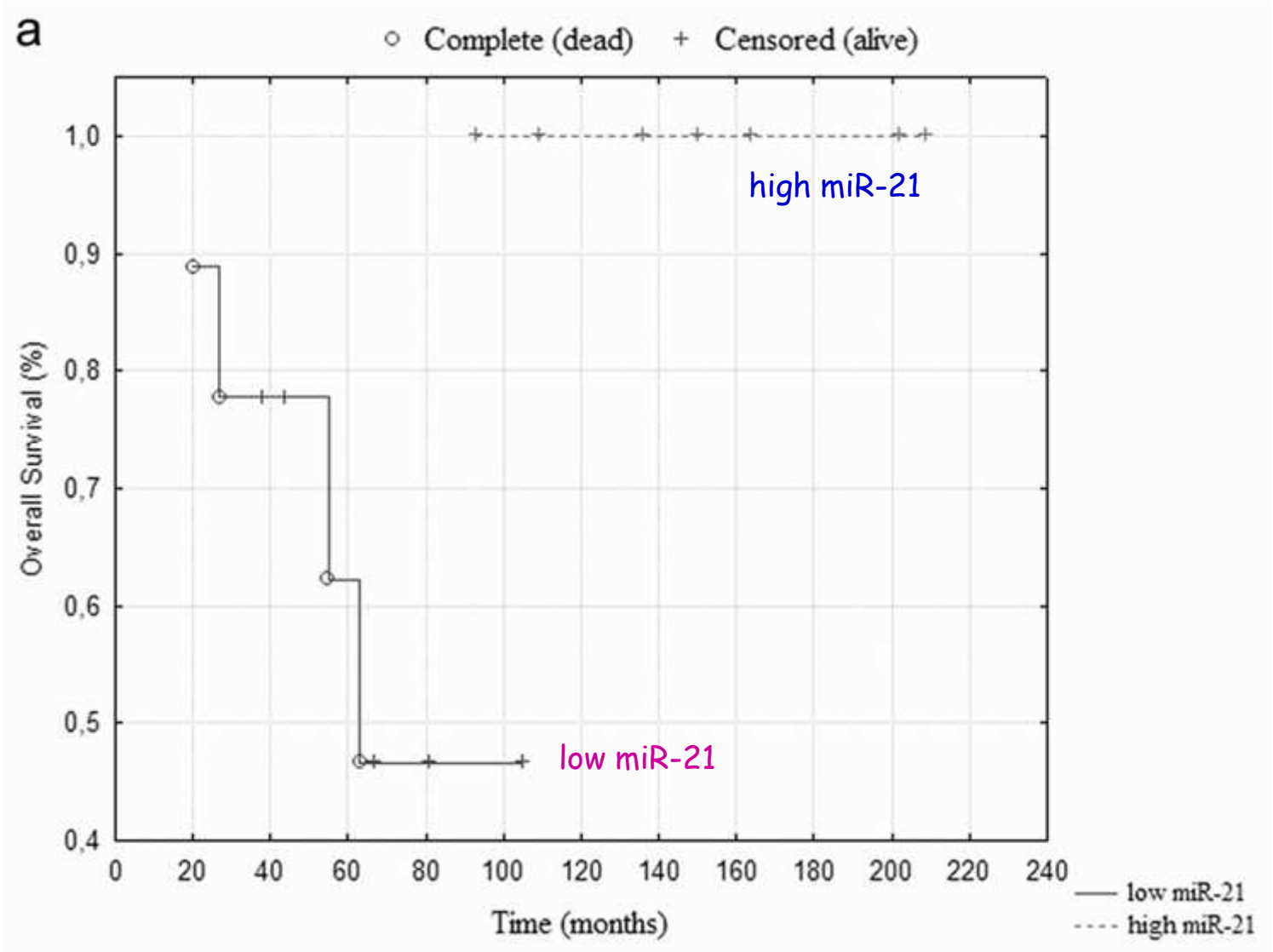
Patient's data indicating the available sample types and clinical outcome.

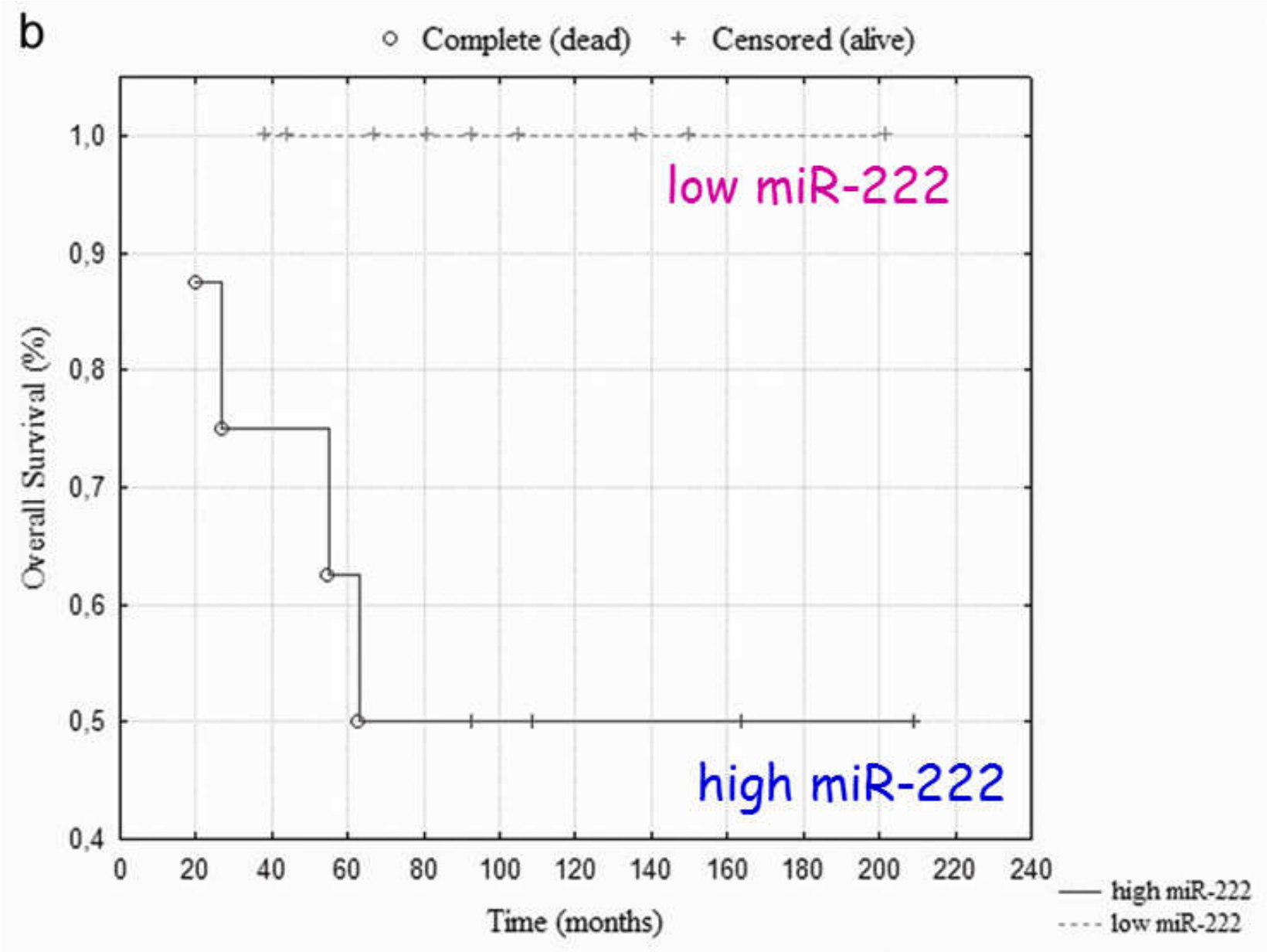
Cases	Gender	Age (year)	Tumor compartment n=23		Surrounding Liver	Sample/patient	Metastasis	Recidive	Death
1	male	0.36	F			1		Yes	Yes
2	female	1.44		E	SL	2			
3	male	10.14	F		SL	2		Yes	
4	male	5.14	F		SL	2	Lung	Yes	Yes
5	female	5.44	F		SL	2			
6	female	0.30	F		SL	2			
7	male	6.45	F		SL	2			
8	female	2.16	F	E		2			
9	male	3.12	F		SL	2			
10	male	2.40		E		1			
11	male	0.76	F		SL	2			
12	male	0.98	F		SL	2	Lung	Yes	
13	female	1.13	F	E		2			
14	male	7.66	F		SL	2			
15	female	4.76		E		1	Lung	Yes	Yes
16	female	15.82	F		SL	2	Lung	Yes	Yes
17	female	1.19		E	SL	2			
18	female	1.54	F		SL	2	Lung		
19	female	0.98		E	SL	2			
20	male	2.16	F	E	SL	3	Lung	Yes	Yes
Total:			**15**	**8**	**15**	**38**	**6**	7	**5**

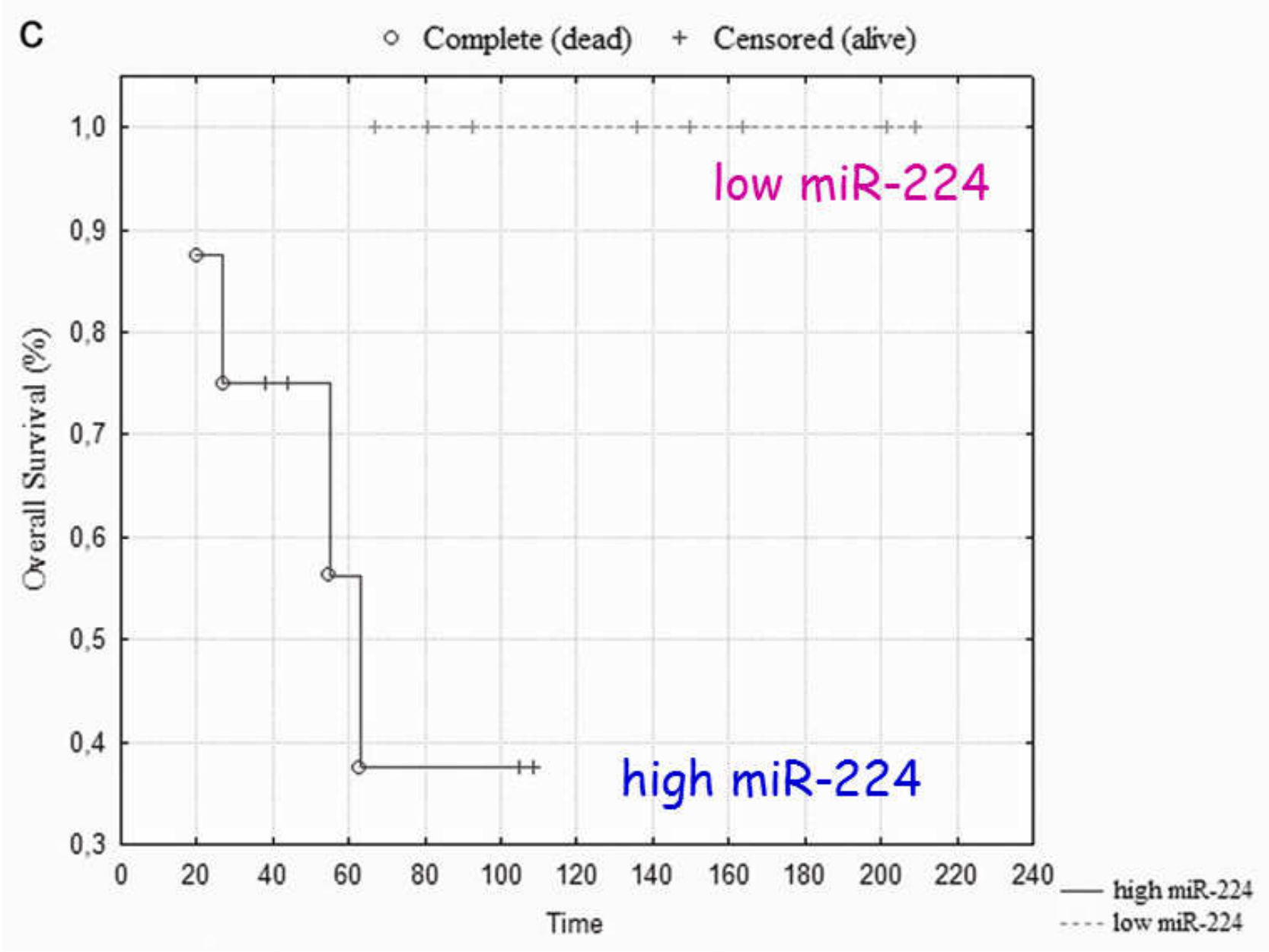
F: fetal, E: embryonal, SL: surrounding liver

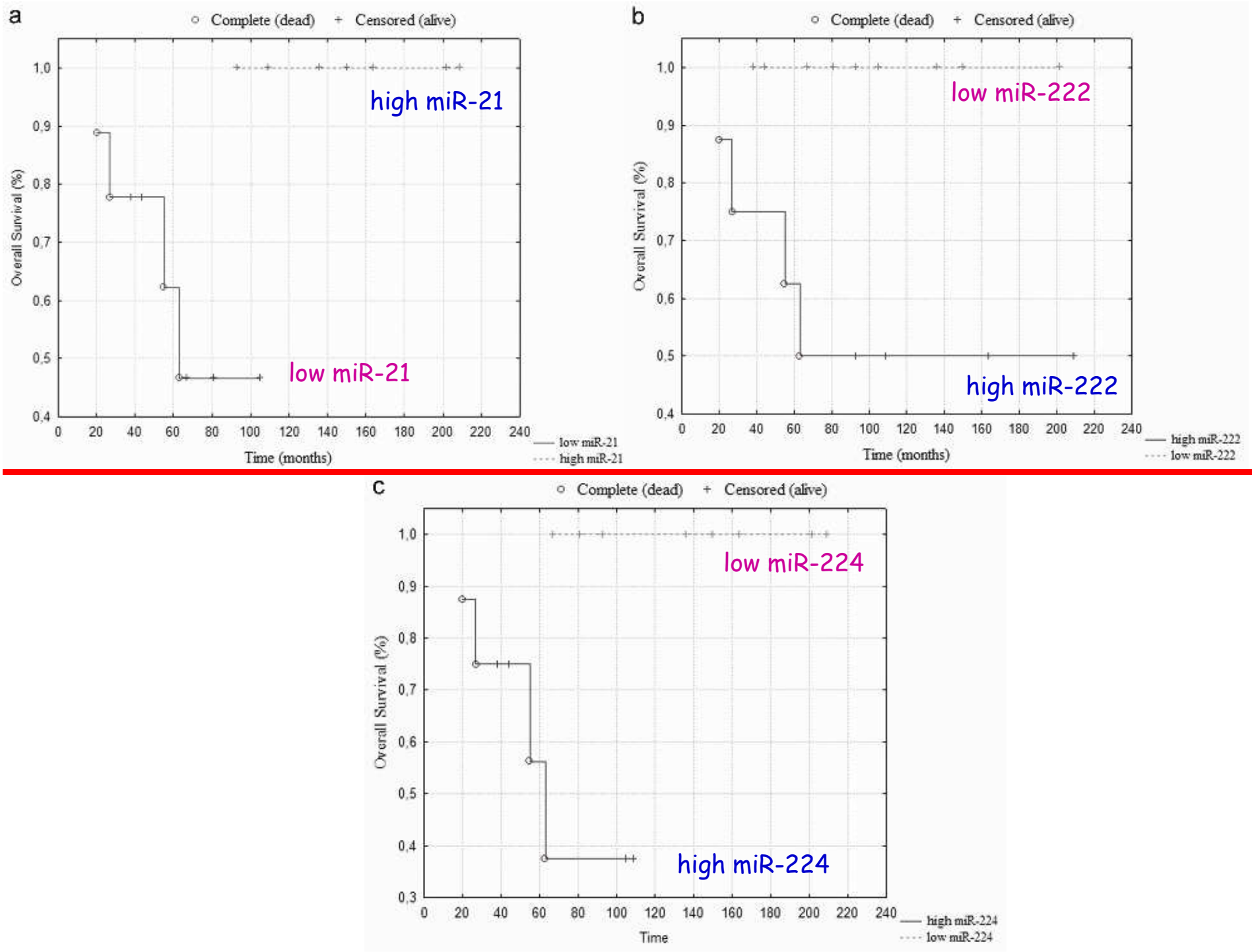

Relative expression
1000
100
10
1
0.1
0.01
HB
SL
miR-17-5p
miR-122
miR-195
miR-210
miR-214
miR-221


Relative expression
1000
100
10
1
0.1
0.01
Embryonal
Fetal
SL
miR-17-5p
miR-18a
miR-122
miR-195
miR-210
miR-214
miR-221


a
Complete (dead)
Censored (alive)
high miR-21
low miR-21
Overall Survival (%)
1,0
0,9
0,8
0,7
0,6
0,5
0,4
0
20
40
60
80
100
120
140
160
180
200
220
240
Time (months)
low miR-21
high miR-21


b
Complete (dead)
Censored (alive)
low miR-222
high miR-222
Overall Survival (%)
1,0
0,9
0,8
0,7
0,6
0,5
0,4
0
20
40
60
80
100
120
140
160
180
200
220
240
Time (months)
high miR-222
low miR-222


C
Complete (dead)
Censored (alive)
low miR-224
high miR-224
Overall Survival (%)
1,0
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0
20
40
60
80
100
120
140
160
180
200
220
240
Time
high miR-224
low miR-224


a
Complete (dead)
Censored (alive)
high miR-21
low miR-21
Overall Survival (%)
Time (months)
low miR-21
high miR-21
b
Complete (dead)
Censored (alive)
low miR-222
high miR-222
Overall Survival (%)
Time (months)
high miR-222
low miR-222
c
Complete (dead)
Censored (alive)
low miR-224
high miR-224
Overall Survival (%)
Time
high miR-224
low miR-224

microRNA expression in HB

- **Histological subtypes of HB did not show correlation with survival.**

- **Higher expression of miR-18a was detected in the embryonal than in the fetal HB component. Further, lower expression of miR-17-5p, miR-122, miR-195, miR-210 and miR-214 and higher expression of miR-221 was found in epithelial components of HB than in SL.**

- **Furthermore, high miR-21 and low miR-222 and miR-224 levels were associated with increased OS of SIOPEL-treated HB patients.**

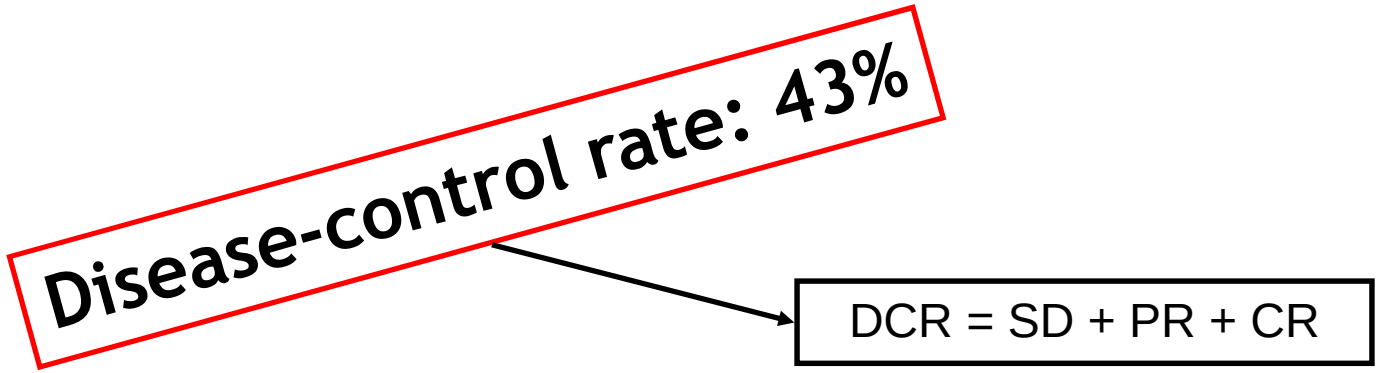
- **These results suggest that miRNA profiling is of potential use as a diagnostic and predictive tool in HB.**

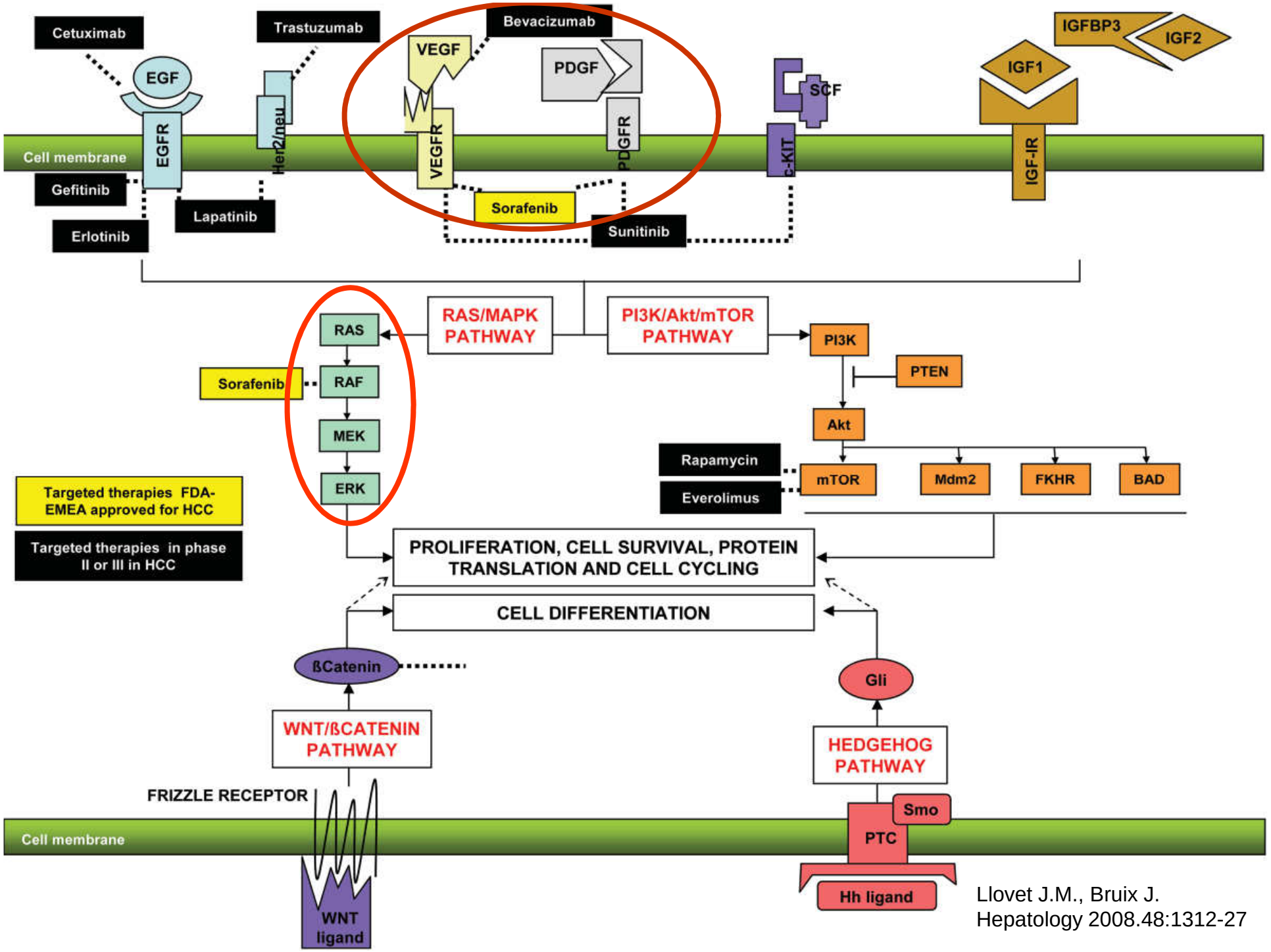
„microRNA expression might predict prognosis of epithelial hepatoblastoma"
Monika Gyugos1, Gábor Lendvai1, István Kenessey1, Krisztina Schlachter1, Judit Halász1, Péter Nagy2, Miklós Garami3, Zsuzsa Jakab3, Zsuzsa Schaff1, András Kiss1#, Virchows Archive, 2014

HCC and sorafenib

- Sorafenib (Nexavar®)
  - Multikinase inhibitor (cRaf, bRaf, VEGFR-2, VEGFR-3, PDGFR-β, c-kit, Flt3)
  - Irresectable cases and in case of recurrence

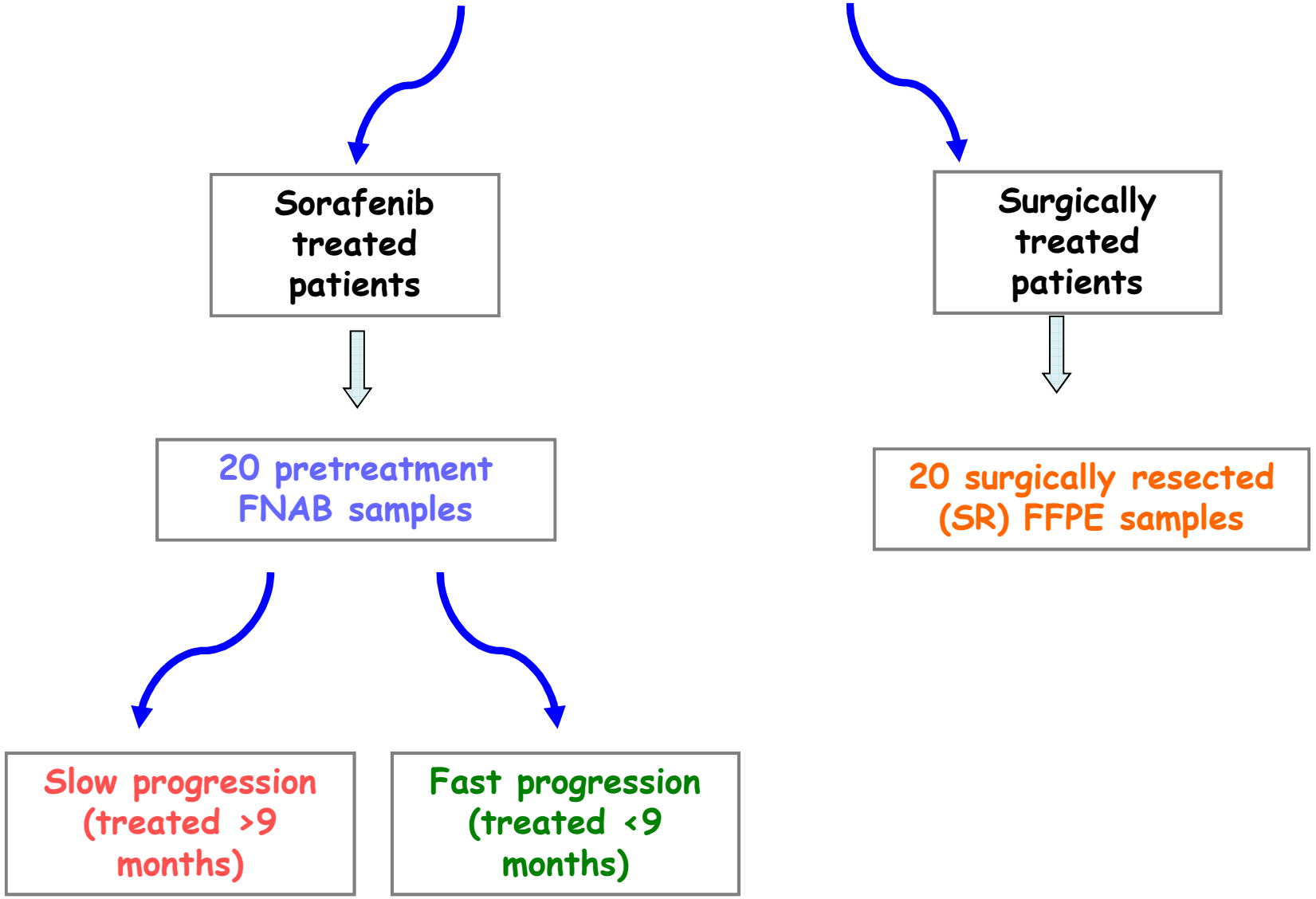
- „In patients with advanced HCC, median survival and the time to radiologic progression were nearly 3 months longer for patients treated with sorafenib than for those given placebo."
  (SHARP trial 2008)





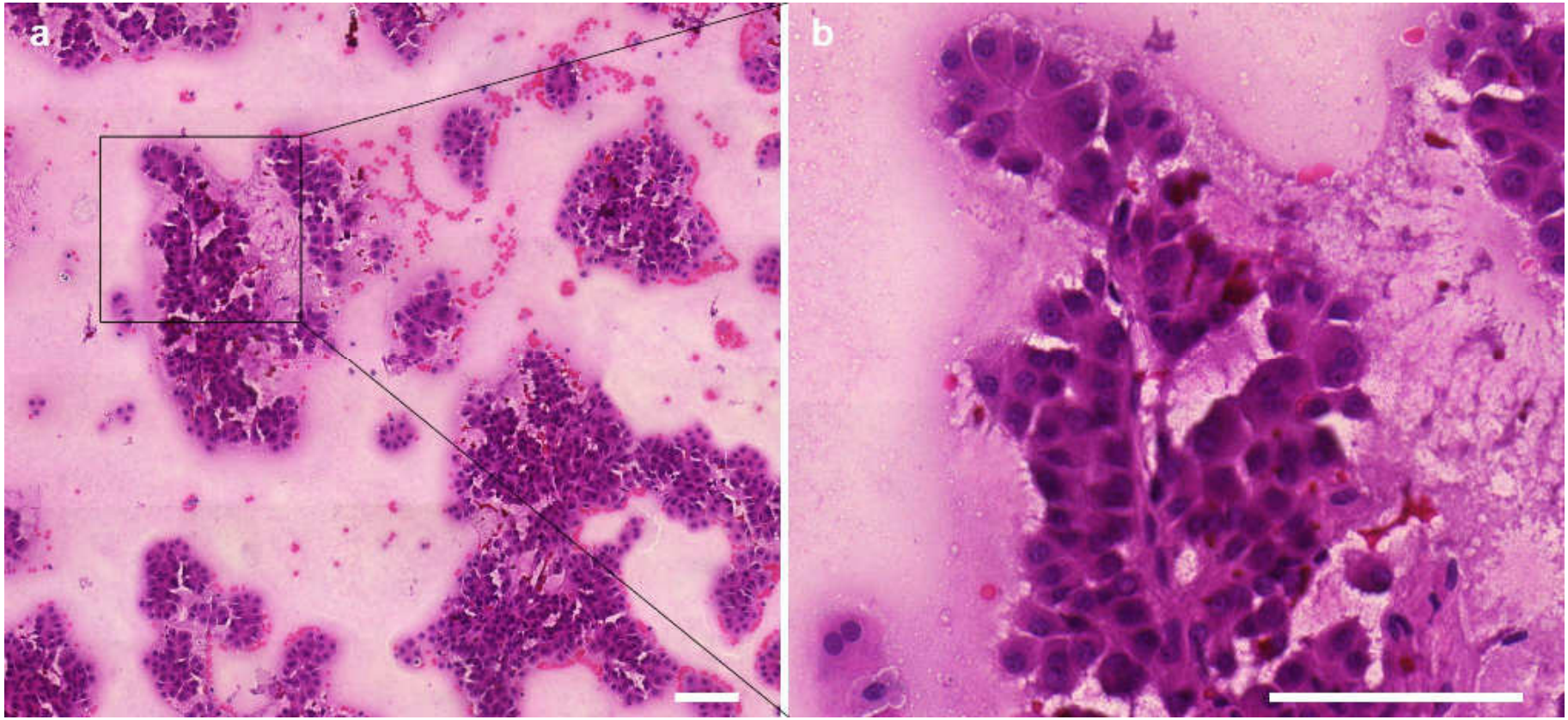
Llovet J.M., Bruix J.
Hepatology 2008.48:1312-27

Patients and material



Sorafenib treated HCC patients and FNAB samples

Twenty sorafenib treated HCC patients (Szt. Istvan – Szt. László Hospital, Oncology Department) pretreatment **FNAB samples** at 2. Dept. of Pathology



Representative image of a hepatocellular carcinoma sample obtained by fine needle aspiration. Irregularly shaped tumor aggregates with transgressing endothelium can be seen. Scale bars 100 mm.

miRNS expression and clinicopathological parameters

Patients were divided into „high ” and „low miR expression” groups according to medián values of relative expression.

Fisher exact teszt p = 0,003		**miR-17-5p**	
		high	low
ECOG	0	10	3
	1	0	7

Increased miR-17-5p expression showed correlation with better ECOG performace status

Fisher exact teszt p = 0,023		**miR-214**	
		high	low
Tumor méret	>5 cm	2	8
	≤5 cm	8	2

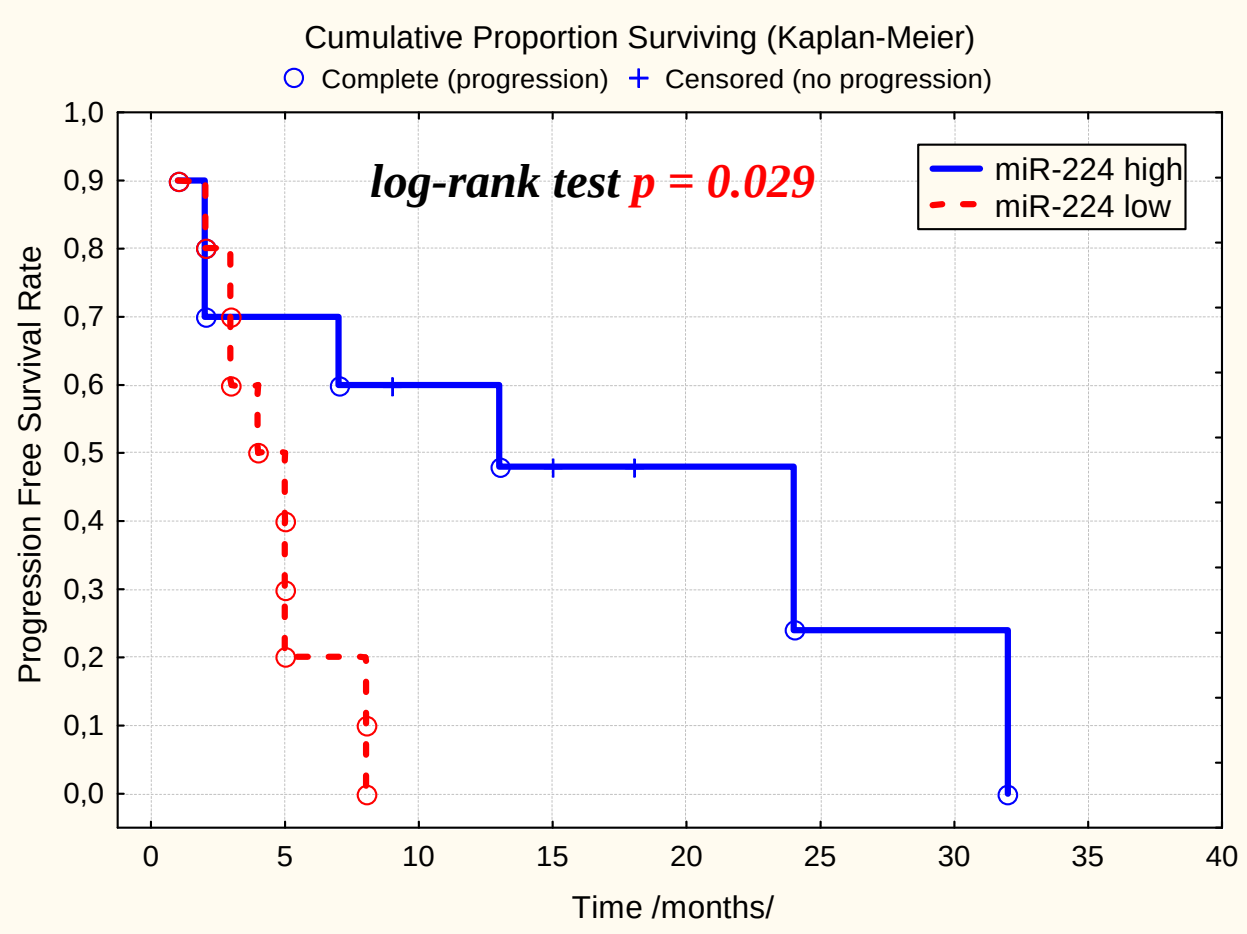
Increased miR-214 expression revealed correlation with smaller tumor size.

→ in corcordance with the literature: miR-214 is a know tumor suppressor, whic inhibits the growth of HCC cells *in vitro*[1].

Reference
1. Wang et al. 2012. Biochem Biophys Res Commun . 428: 525-31.

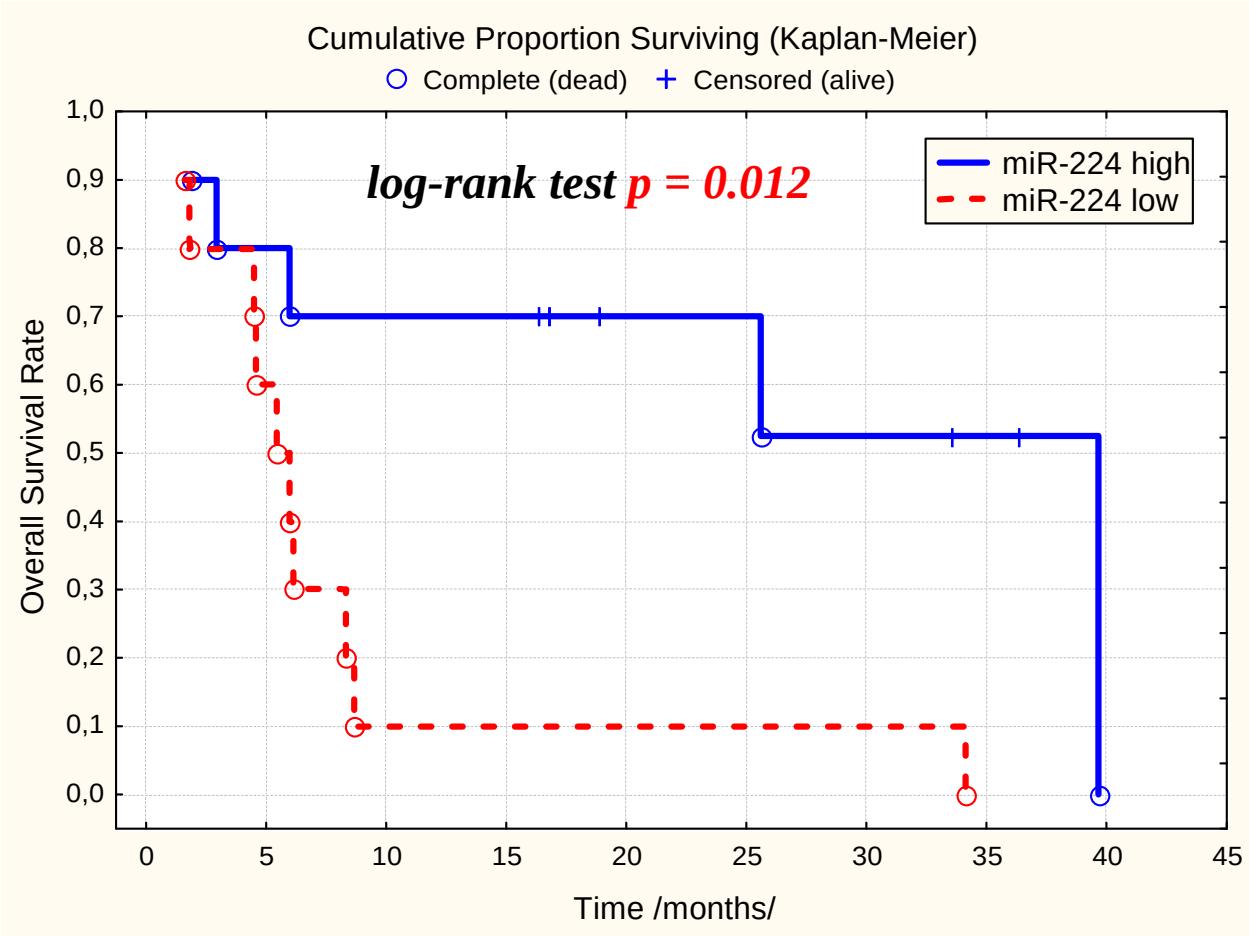
Pretreatment miR-224 expression and disease outcome

Progression-free survival



Pretreatment miR-224 expression and disease outcome

Overall survival



Validated and potential targets of miR-224

- **API5; Bcl2; MAPK1; SMAD4**

  → miR-224 **regulates apoptosis in** HCC cells

- miR-224 is one of the most frequently overexpressed oncomiR in HCC
- Increased miR-224 expression is associated with worse prognosis HCC patients with resected tumor as well as in cervix carcinoma and glioma

→ Putative miR-224 targets are : PDGFR-α and -ß (Dweep et al. 2011, Murakami et al., 2006)

- **PDGFR-ß** → **known target of sorafenib**

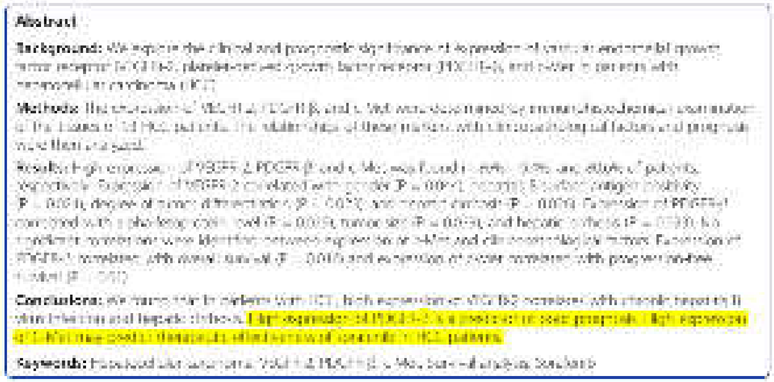
  → Chu et al. 2013. JECCR 32:16

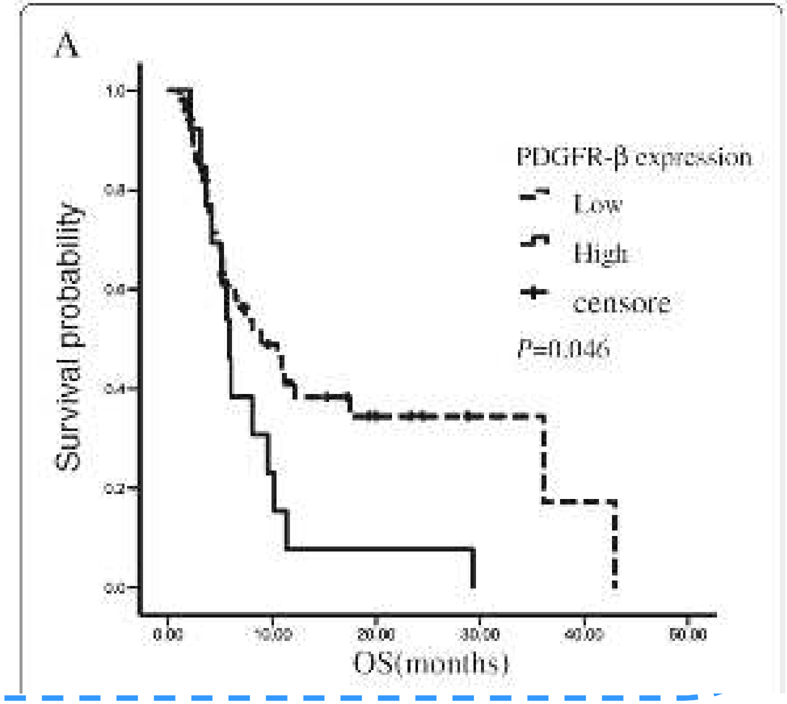
  * biopsy material of 93 HCC patients (65 sorafenib treated)
  * increased **PDGFR-ß expression is associated with poor prognosis**

Chu *et al. Journal of Experimental & Clinical Cancer Research* 2013, **32**:16
http://www.jeccr.com/content/32/1/16

Journal of Experimental &
Clinical Cancer Research

Expression and prognostic value of VEGFR-2, PDGFR-β, and c-Met in advanced hepatocellular carcinoma





Drakaki *et al. BMC Cancer* (2015) 15:542
DOI 10.1186/s12885-015-1562-9

BMC Cancer

RESEARCH ARTICLE Open Access

Functional microRNA high throughput screening reveals miR-9 as a central regulator of liver oncogenesis by affecting the PPARA-CDH1 pathway

Alexandra Drakaki[1,2], Maria Hatziapostolou[3], Christos Polytarchou[3], Christina Vorvis[3], George A. Poultsides[4], John Souglakos[2], Vassilis Georgoulias[2] and Dimitrios Iliopoulos[3*]

Abstract

Background: Hepatocellular carcinoma (HCC) is the second leading cause of cancer-related deaths, reflecting the aggressiveness of this type of cancer and the absence of effective therapeutic regimens. MicroRNAs have been involved in the pathogenesis of different types of cancers, including liver cancer. Our aim was to identify microRNAs that have both functional and clinical relevance in HCC and examine their downstream signaling effectors.

Methods: MicroRNA and gene expression levels were measured by quantitative real-time PCR in HCC tumors and controls. A TargetScan algorithm was used to identify miR-9 downstream direct targets.

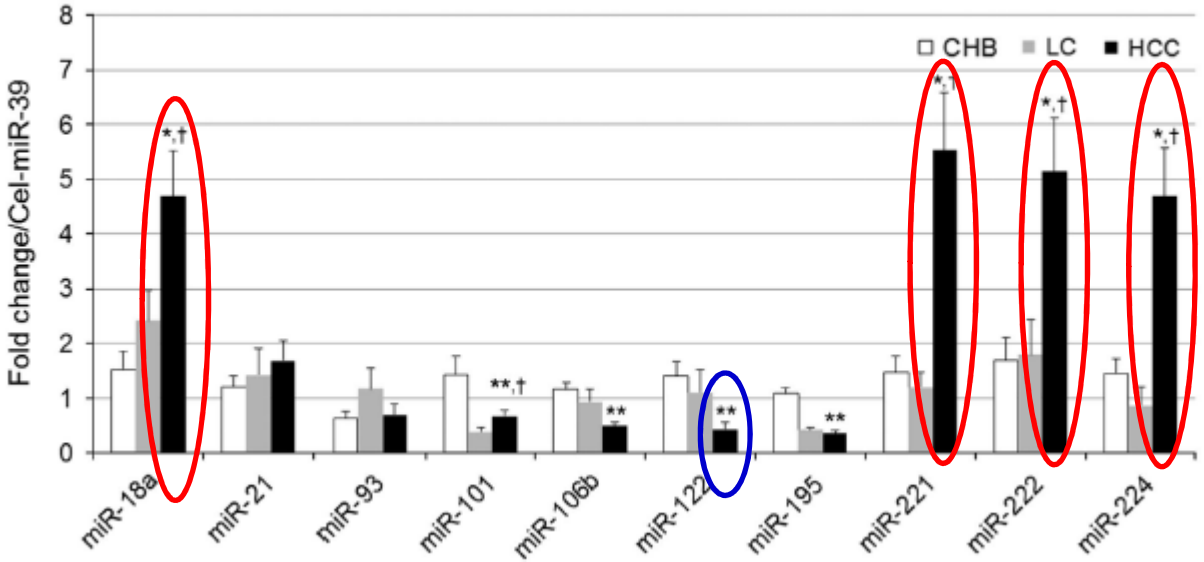
Results: A high-throughput screen of the human microRNAome revealed 28 microRNAs as regulators of liver cancer cell invasiveness. MiR-9, miR-21 and miR-224 were the top inducers of HCC invasiveness and also their expression was increased in HCC relative to control liver tissues. Integration of the microRNA screen and expression data revealed miR-9 as the top microRNA, having both functional and clinical significance. MiR-9 levels correlated with HCC tumor stage and miR-9 overexpression induced SNU-449 and HepG2 cell growth, invasiveness and their ability to form colonies in soft agar. Bioinformatics and 3'UTR luciferase analyses identified E-cadherin (CDH1) and peroxisome proliferator-activated receptor alpha (PPARA) as direct downstream effectors of miR-9 activity. Inhibition

Serum exosomal microRNAs as novel biomarkers for hepatocellular carcinoma

Won Sohn[1,4], Jonghwa Kim[1,4], So Hee Kang[1], Se Ra Yang[1], Ju-Yeon Cho[1], Hyun Chin Cho[2], Sang Guon Shim[2] and Yong-Han Paik[1,3]

Recent studies have shown that circulating microRNAs are a potential biomarker in various types of malignancies. The aim of this study was to investigate the feasibility of using serum exosomal microRNAs as novel serological biomarkers for hepatocellular carcinoma (HCC) in patients with chronic hepatitis B (CHB). We measured the serum exosomal microRNAs and serum circulating microRNAs in patients with CHB ($n = 20$), liver cirrhosis (LC) ($n = 20$) and HCC ($n = 20$). Serum exosomal microRNA was extracted from 500 μl of serum using an Exosome RNA Isolation kit. The expression levels of microRNAs were quantified by real-time PCR. The expression levels of selected microRNAs were normalized to *Caenorhabditis elegans* microRNA (*Cel-miR-39*). The serum levels of exosomal miR-18a, miR-221, miR-222 and miR-224 were significantly higher in patients with HCC than those with CHB or LC ($P < 0.05$). Further, the serum levels of exosomal miR-101, miR-106b, miR-122 and miR-195 were lower in patients with HCC than in patients with CHB ($P = 0.014$, $P < 0.001$, $P < 0.001$ and $P < 0.001$, respectively). There was no significant difference in the levels of miR-21 and miR-93 among the three groups. Additionally, the serum levels of circulating microRNAs showed a smaller difference between HCC and either CHB or LC. This study suggests that serum exosomal microRNAs may be used as novel serological biomarkers for HCC.

Experimental & Molecular Medicine (2015) 47, e184; doi:10.1038/emm.2015.68; published online 18 September 2015

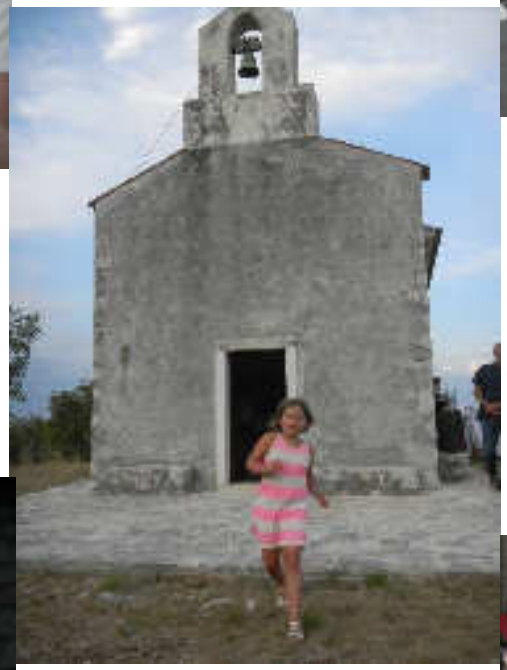


Conclusions

- microRNAs could be investigated in archive FNAB cytology samples
- microRNAs are potential predictive factors

- **Pretreatment miR-224 level might bear with predictive value regarding to therapeutic response in HCC patients treated with sorafenib**.

Thank you attention !



for your

Thank you for your attention !

Prof. Dr. Zsuzsa Schaff

- Dr. Gyöngyösi Benedek
- Dr. Gábor Lendvai
- Dr. Balázs Járay
- Dr. Eszter Székely
- Dr. Krisztina Schlachter
- Monika Gyugos
- Dr. István Kenessey

- Prof. Dr. László Harsányi
- Prof. Dr. Péter Kupcsulik
- Dr. Attila Szijártó
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