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 Klinik und Poliklinik für  
**UROLOGIE**  
und Kinderurologie

# Die Bedeutung von long non-coding RNAs beim Nierenzellkarzinom

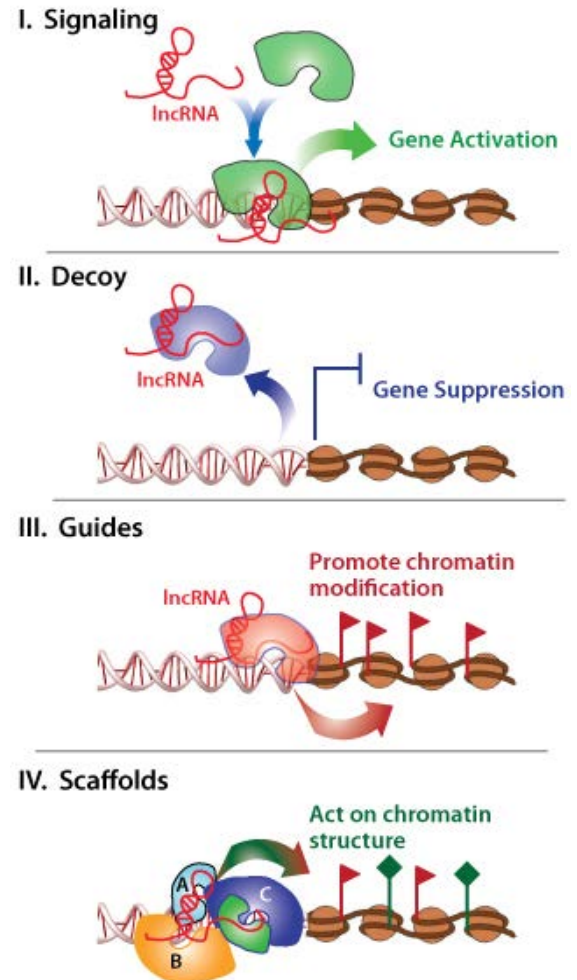
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# Long non-coding RNAs

- » Definition: nicht für Protein kodierend, Größe > 200 nt
- » LNCpedia v3.1: 111.685 lncRNAs
- » Expression:
  - dynamisch, kurze Halbwertszeiten
  - eher niedrige Expressionslevel
  - Gewebe-/Tumor-spezifisch
- » Beeinflussung zellulärer Prozesse durch Modulation der Expression anderer Gene



# Studien-Design

## Screening

- Microarray (32.183 lncRNA Transkripte)
- 15 ccRCC vs. Normalgewebe

## Validierung

- qPCR (12 lncRNAs)
- 55 RCC vs. 52 CTRL / 102 RCC vs. 50 CTRL

## Funktionelle Untersuchungen

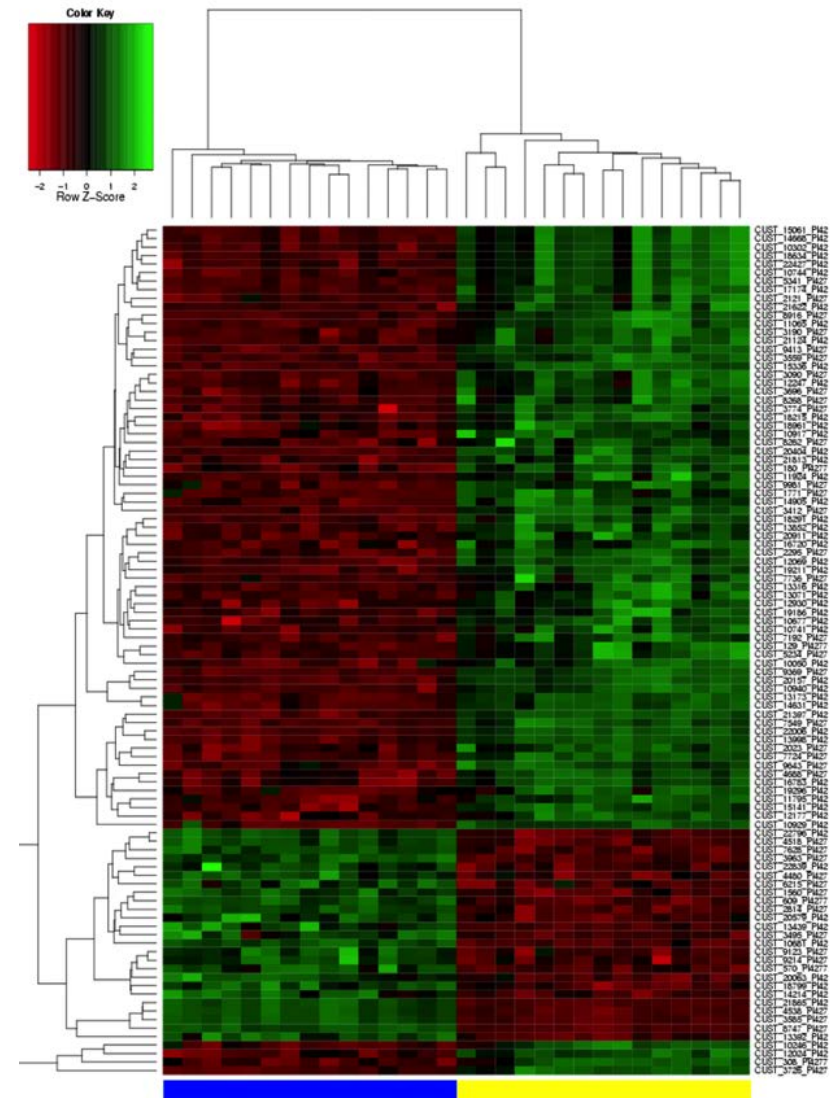
- siRNA Knockdown
- Bioinformatische Analysen

# Microarray - IncRNA Expressionsprofiling: Vergleich ccRCC vs. Normalgewebe

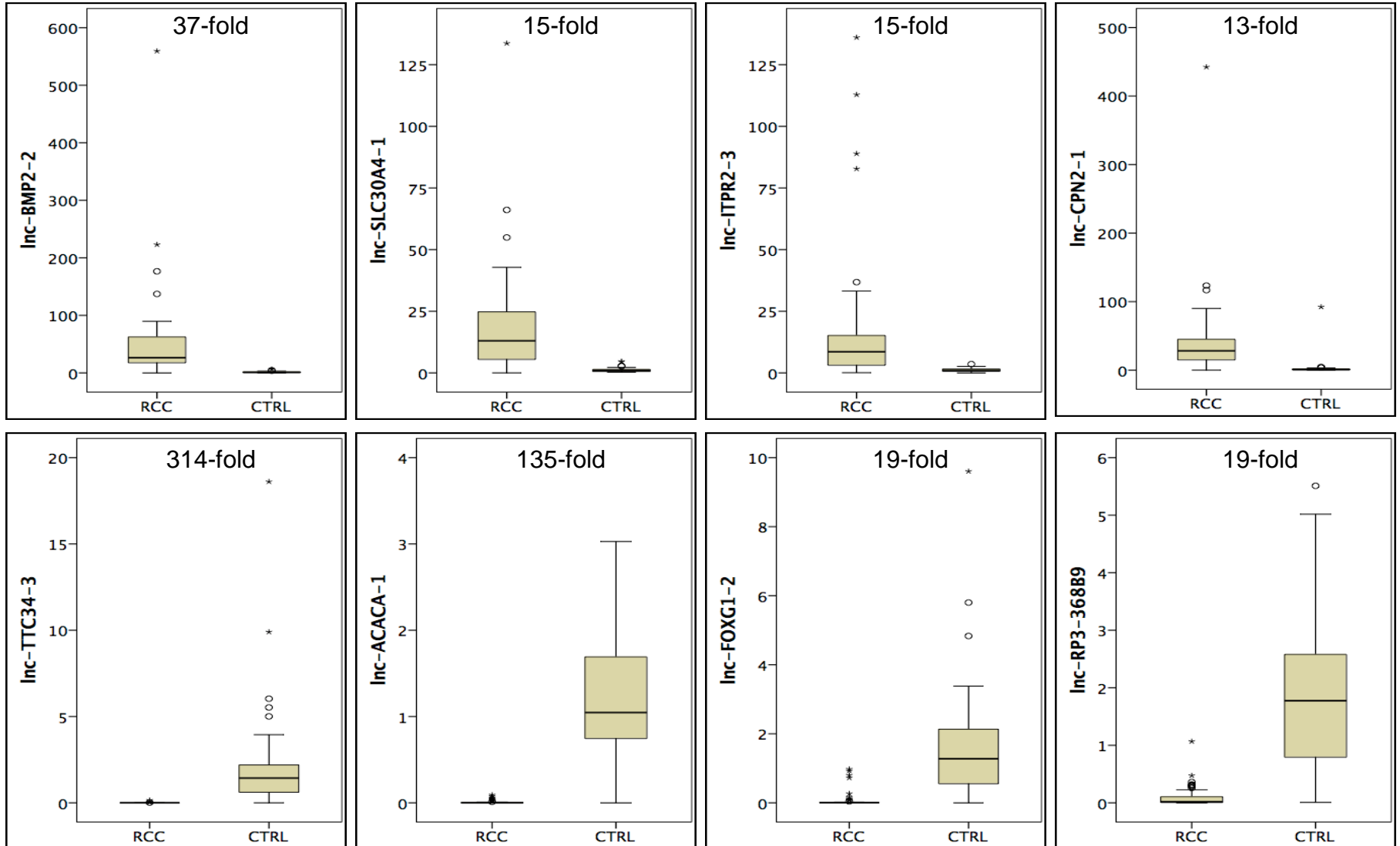
## » Differentielle Expression \*

- 1.308 IncRNA Transkripte
- 740 Down-Regulated
- 568 Up-Regulated

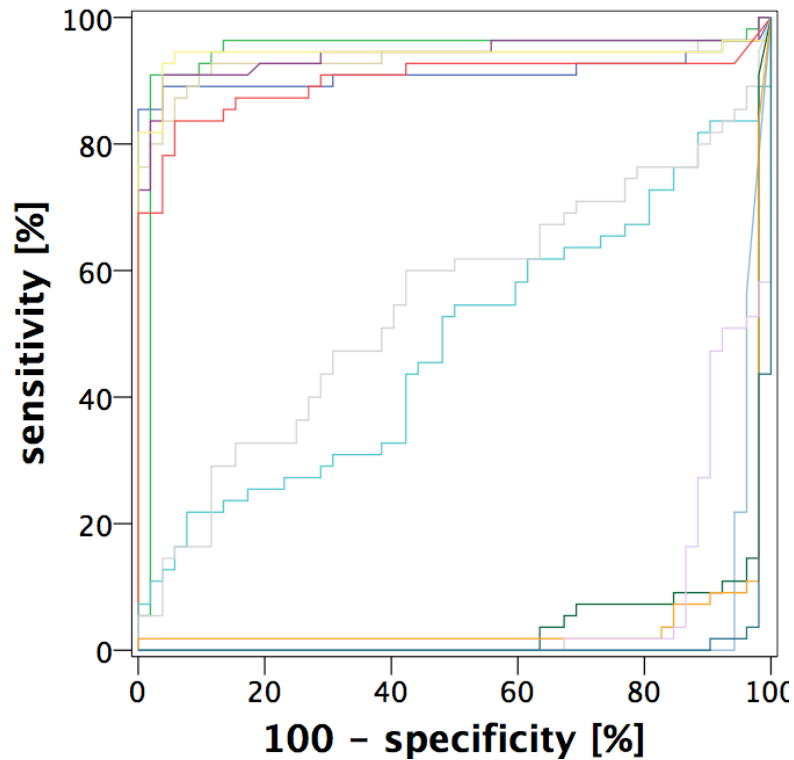
\* 2-fold expression difference



# qPCR: Validierung differenzielle IncRNA Expression in Tumor und Normalgewebe



# qPCR: Validierung differenzielle IncRNA Expression in Tumor und Normalgewebe



- Inc-ERCC5-1
- Inc-ACACA-1
- Inc-FOXG1-2
- Inc-LCP2-2
- Inc-RP3-368B9
- Inc-TTC34-3
- Inc-BMP2-2
- Inc-CPN2-1
- Inc-FZD1-2
- Inc-ITPR2-3
- Inc-SLC30A4-1
- Inc-RP11-480I12.4.1-1

IncRNA	AUC	95% CI
Inc-FZD1-2	0.931	0.871 - 0.991
Inc-SLC30A4-1	0.942	0.883 - 1.000
Inc-BMP2-2	0.912	0.843 - 0.981
Inc-SPAM1-6	0.900	0.830 - 0.969
Inc-ITPR2-3	0.941	0.887 - 0.994
Inc-CPN2-1	0.942	0.884 - 1.000
Inc-TTC34-3	0.990	0.973 - 1.000
Inc-ACACA-1	0.966	0.923 - 1.000
Inc-LCP2-2	0.955	0.906 - 1.000
Inc-FOXG1-2	0.954	0.911 - 0.997
Inc-RP3-368B9.1	0.938	0.892 - 0.984

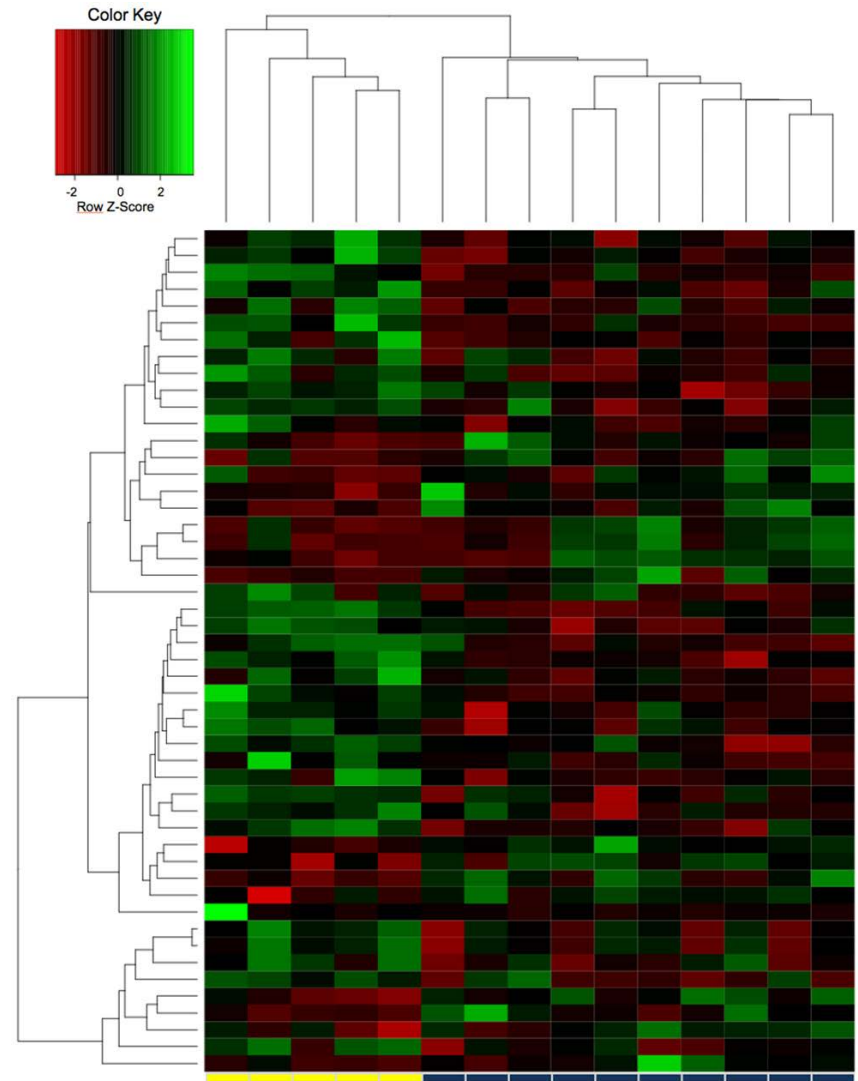
# Microarray - IncRNA Expressionsprofiling: Vergleich lokal-begrenzte vs. fortgeschrittene ccRCC

» Vergleich Gewebe:  
5 lokal-begrenzte vs.  
10 fortgeschrittene RCC

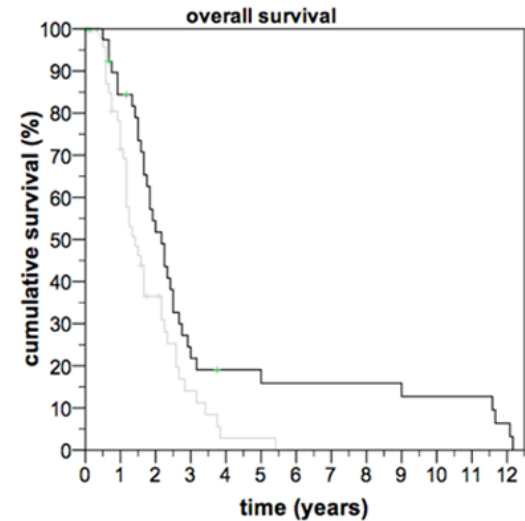
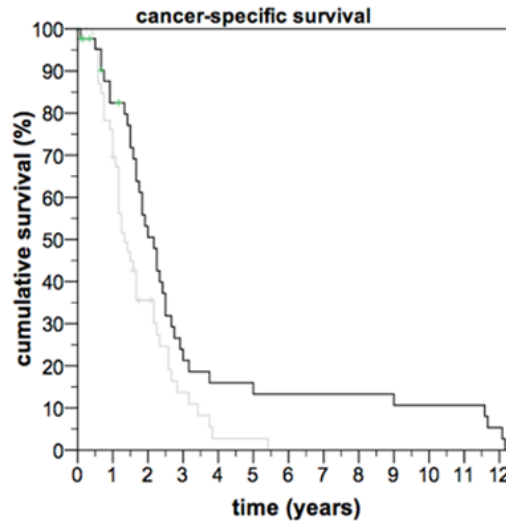
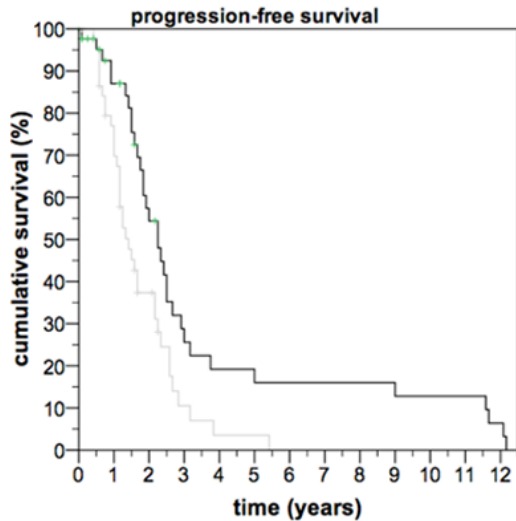
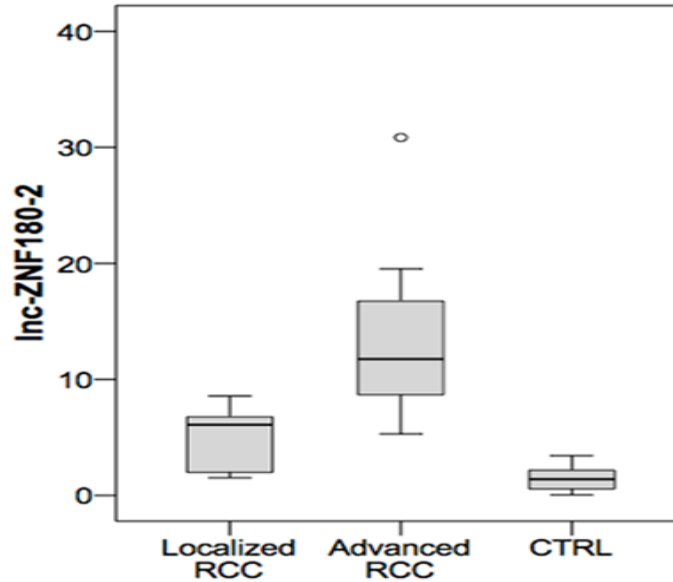
» Differentielle Expression \*

- 49 IncRNA Transkripte
- 32 Down-Regulated
- 17 Up-Regulated

\* 2-fold expression difference



# qPCR: Validierung differenzielle IncRNA Expression in lokal-begrenztem und fortgeschrittenem ccRCC



— Inc-ZNF180-2 < median — Inc-ZNF180-2 > median



# Bioinformatische Untersuchungen: lncRNA-Protein-Interaktionen

## » catRAPID Omics:

Vorhersage von Interaktionen (Score >2.5) mit 334 Proteinen für lnc-BMP2-2, lnc-CPN2-1, lnc-FZD1-2, lnc-ITPR2-3, lnc-SLC30A4-1, lnc-SPAM1-6, lnc-ACACA-1, lnc-FOXG1-2, lnc-LCP2-2, lnc-RP3-368B9, lnc-TTC34-3

## » GeneMANIA:

Predicted function	lnc-ACACA-1	lnc-BMP2-2	lnc-CPN2-1	lnc-FOXG1-2	lnc-FZD1-2	lnc-ITPR2-3	lnc-LCP2-2	lnc-RP3-368B9.1.1-1	lnc-SLC30A4-1	lnc-SPAM1-6	lnc-TTC34-3
mRNA processing	X	X	X	X	X	X	X	X	X	X	X
mRNA/RNA splicing					X	X	X	X	X	X	X
mRNA binding		X	X	X	X	X					X
RNA localization	X	X				X	X	X	X	X	X
Ribonucleoprotein complex		X			X	X	X	X	X		X
RNA transport	X					X	X	X	X	X	X
Transcription	X					X	X	X	X	X	X
Posttranscriptional regulation		X	X	X	X	X	X				
mRNA/RNA stabilization				X	X	X					
DNA binding						X				X	X
Translation					X		X				
Protein localization									X		
rRNA processing									X		
Viral genome expression									X		

**RNA transcription**

**RNA stabilization**

**mRNA binding**

**RNA localization**

**mRNA splicing**

**mRNA processing**

**posttranscriptional regulation**

# Bioinformatische Untersuchungen: lncRNA – microRNA Interaktionen

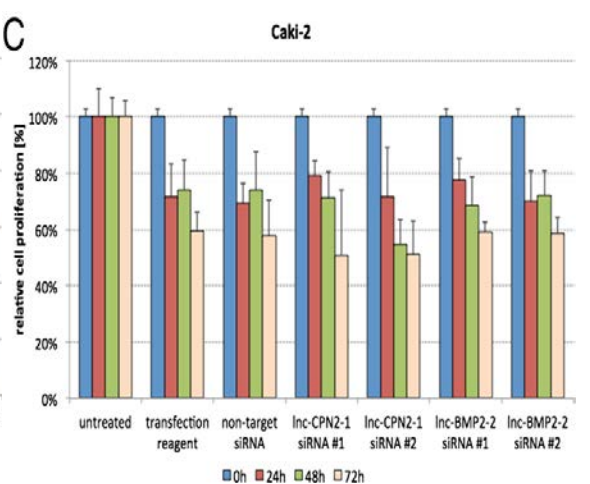
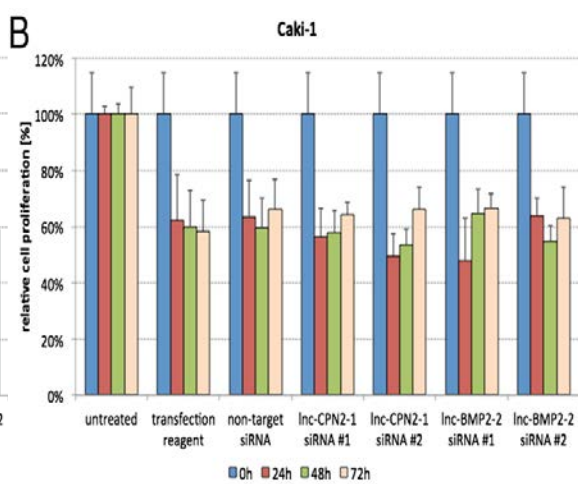
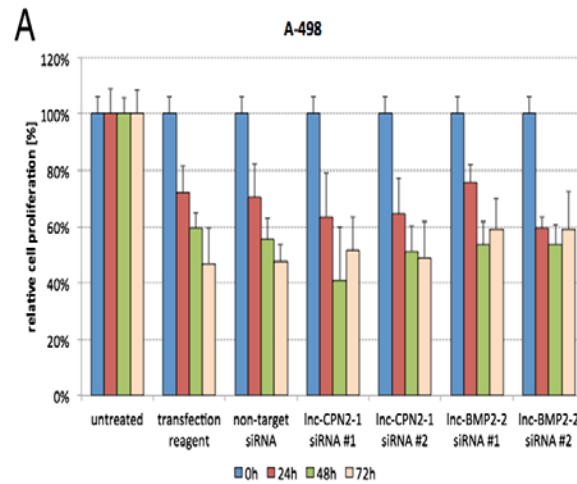
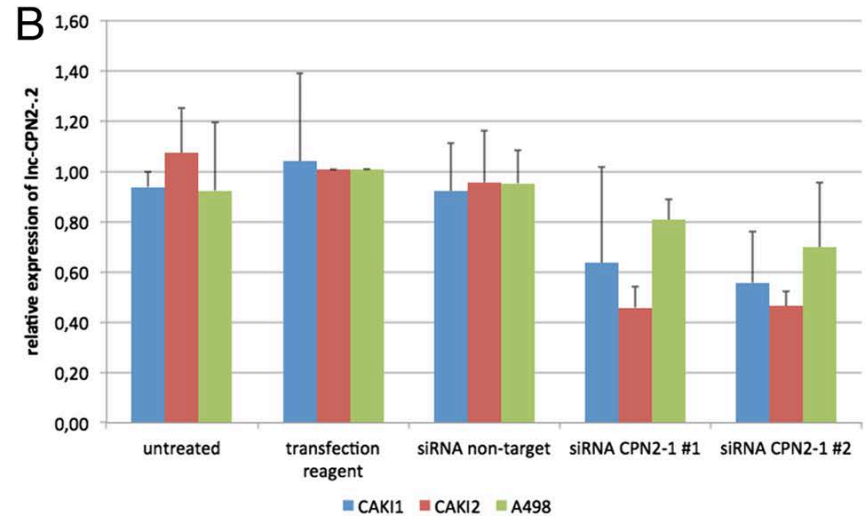
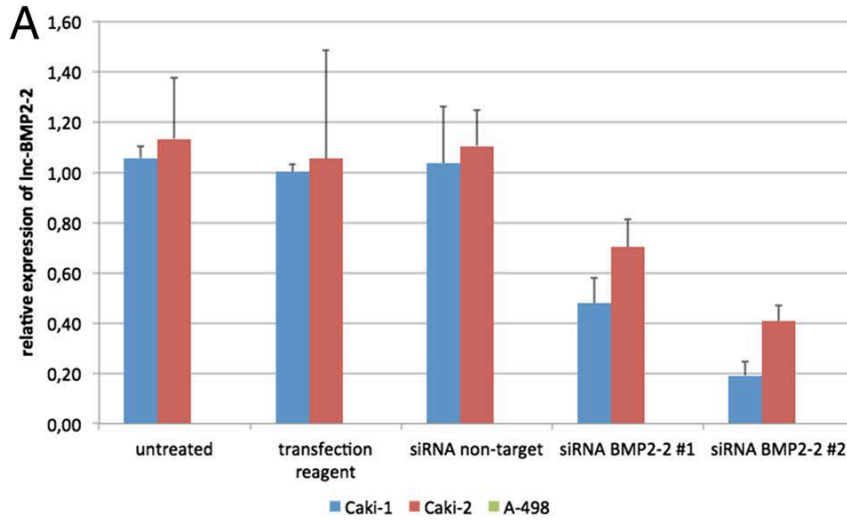
## » mirTarget2:

- Vorhersage von Interaktionen von lnc-FOXP1-2 mit 12 microRNAs:  
miR-3662, miR-3120-5p, miR-519c-3p, miR-519a-3p, miR-548ag, miR-4282, miR-519b-3p, miR-458, miR-3163, miR-4658, miR-676-5p, miR-3973

## » Validierung mittels qPCR:

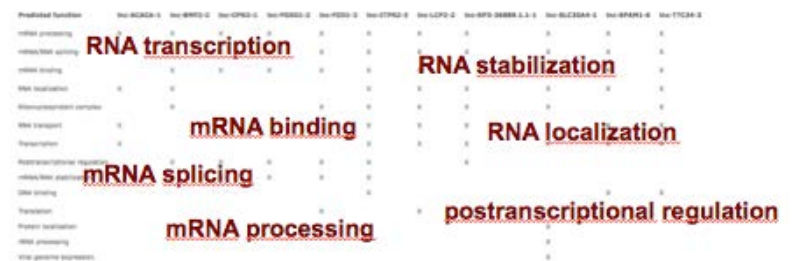
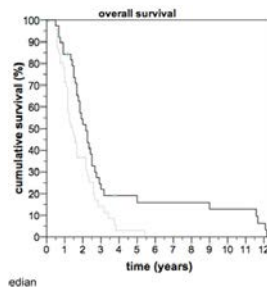
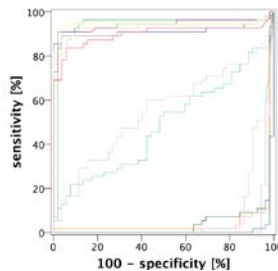
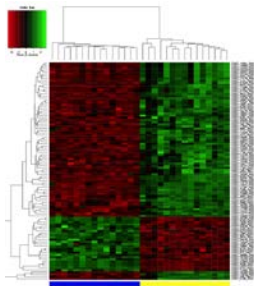
- je 10 Normal und 10 ccRCC Gewebe
- keine Korrelation von lnc-FOXP1-2 mit miRNA Expression

# siRNA Knockdown: Inc-BMP2-2 und Inc-CPN2-2



# Zusammenfassung

- » IncRNA Expressionsprofil in ccRCC
- » diagnostisches Potential einzelner IncRNA (Inc-ACACA-1)
- » prognostisches Potential (Inc-ZNF180-2)
- » Funktionsweise: Modulation anderer Proteine



## Urologie

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Jahedul Alam

Stefan C. Müller

## Pathologie

Mario Deng

Sven Perner

## Biobank

Carsten Golletz

Makbule Kobilay

Stefan Holdenrieder

1. Blondeau et al.: Identification of novel long non-coding RNAs in clear cell renal cell carcinoma. *Clinical Epigenetics* 2015.
2. Deng et al.: Identification of novel differentially expressed lncRNA and mRNA transcripts in clear cell renal cell carcinoma by expression profiling. *Genomics Data* 2015.
3. Ellinger et al.: The long non-coding RNA Inc-ZNF180-2 is a prognostic biomarker in patients with clear cell renal cell carcinoma, *American Journal of Cancer Research* 2015.