The PDGFRα-Laminin B1-Keratin19 cascade drives tumor progression at the invasive front of human hepatocellular carcinomas

Supplementary data

Olivier Govaere¹² *, Michaela Petz³ *, Jasper Wouters¹, Yves-Paul Vandenbroucke⁴, Emma J. Scott², Baki Topal⁵, Frederik Nevens⁶, Chris Verslype⁶, Quentin M. Anstee², Hans Van Vlierberghe⁶, Wolfgang Mikulits³ψ, Tania Roskams¹ψ.

* These authors contributed equally.

Supplementary Table S1. Clinical and pathological features of HCC needle biopsy cohort (n=46).

|                        | PDGFRα positive | PDGFRα negative | p-value |
|------------------------|-----------------|-----------------|---------|
| Immunoreactivity       | 65.22% (30/46)  | 34.78% (16/46)  |         |
| Age (years) (mean)     | 64.50           | 65.8            | na      |
| Gender                 | female 76.67% (23/30) | male 75% (12/16) |         |
| Antigen                |                 |                 |         |
| HBV                   | 3.33% (1/30)    | 31.25% (5/16)   |         |
| HCV                   | 13.33% (4/30)   | 6.25% (1/16)    |         |
| ALD                   | 0% (0/30)       | 0% (0/16)       |         |
| ALD + viral infection | 8.67% (3/35)    | 0% (0/16)       |         |
| Other                  |                 |                 |         |
| Microvascular Invasion | 33.33% (10/30)  | 6.25% (1/16)    | p=0.068 |
| Metastasis             |                 |                 |         |
| K19 expression         | 30% (9/30)      | 0% (0/16)       | p=0.018 |
| Differentiation grade  |                 |                 |         |
| Well                   | 26.67% (8/30)   | 56.25% (9/16)   |         |
| Moderately different   | 43.33% (13/30)  | 37.5% (6/16)    |         |
| Poorly                 | 30% (9/30)      | 6.25% (1/16)    |         |
| Cirrhosis              |                 |                 |         |
| No                     | 10% (3/30)      | 12.5% (2/16)    |         |
| Yes                    | 83.33% (25/30)  | 87.5% (14/16)   |         |
| Unknown                |                 |                 |         |

Supplementary Table S2. Regulatory elements for KRT19 obtained from http://www.genecards.org/cgi-bin/carddisp.pl?gene=KRT19 using the GeneHancer.
### Supplementary Table S3. Primer pairs for PCR and quantitative PCR.

| PCR transcript | forward primer | reverse primer |
|----------------|----------------|----------------|
| ITGA1          | ggttaccctgtgctgtaccc | aagcactcagcaggatgacc |
| ITGA2          | ggtgctttcctgagaaccga | tgggatgtctgggatgttgc |
| ITGA3 A        | atggcaagtggctgctgtat | agcgggtccgcttaaagaag |
| ITGA3 B        | atggcaagtggctgctgtat | agcgggtccgcttaaagaag |
| ITGA6 1        | gggctcattcagcggtcg | cgtggggtcagcatcgttat |
| ITGA6 2        | gggctcattcagcggtcg | cgtggggtcagcatcgttat |
| ITGB1 1A       | gcgcggaaaagatgaatttacaa | tcctgagcttagctggtgttg |
| ITGB4 1        | gggctcattcagcggtcg | cgtggggtcagcatcgttat |
| ITGB4 2        | gggctcattcagcggtcg | cgtggggtcagcatcgttat |
| ITGB4 3        | gggctcattcagcggtcg | cgtggggtcagcatcgttat |
| PDGF A -       | ggcttgcctgctgctcctcg | ttccaccttggccaccttgac |
| PDGF B -       | tgctgagtgaccactcgatc | gactcgcaccgtccgaatgg |
| PDGFR A -      | gagatcatttgggaaggccg | gtaccaccccctcactgttg |
| PDGFR B -      | cgctgaacgtggtcaacctgtt | ggcattgtagaactgctcgt |
| LAMB1 -        | ggcgtcttctccactcctct | gcatagcagctggacggaat |
| RhoA m/h       | ggaagaaactggtgattgttggtg | tcgtggttggcttctaaatactgg |
| RhoA h         | ggctggttactatggcgac | gcacagtcgtcacatctgga |
| KRT19 -        | ccatcatcctggttgggaat | ccatgtacccaaagcgc |

#### Supplementary Figure S1. Sequential immunohistochemical stainings for La/SSB, LAMB1 and K19 on human HCCs diagnosed with microvascular invasion (n=15).

(A) Representative HCC sample showing strong cytoplasmic positivity for La/SSB and LAMB1 at the invasive front (arrow) compared to the surrounding tissue (asterix). (B) Expression of LAMB1 and K19 is strongly expressed at the invasive front of human HCCs. Scale bar 200 µm.
**Supplementary Figure S2.** (A) Immunocytochemistry for PDGFRα on human hepatoma cell lines (magnification 400x). (B) Western blot and (C) PCR analysis of PDGF receptor expression in HCC cell lines.

**Supplementary Figure S3.** Transcriptional regulators of KRT19. (A) TEAD4 was identified as a potential transcriptional regulator by comparing candidate regulatory elements (obtained using GeneHancer) with a previously published HCC data set of KRT19-associated genes. (B) *In silico* ChIP data analysis identified KRT19 as a binding site for TEAD4 in HepG2 cells. (C) Network of TEAD4-KRT19 associated genes (D) Ln-111 coating induced a translocation of TEAD4 and YAP1 from the cytoplasm to the nucleus in HepG2 (t=24h) and in SNU-423 with stable knockdown of Laminin B1 (t=5h).
Supplementary Figure S4. Western blot of Keratin 19 in HepG2 and SNU-423 cell lines. Cells were cultured for 24 hours on plastic or collagen I (BD Biosciences, USA, 354236).

Supplementary Figure S5. Prognostic value of the *ITGA2-ITGB1-TEAD4-KRT19* gene signature. (A) *In silico* analysis shows that the four gene signature shows a significant tendency towards co-occurrence. (B) The *ITGA2-ITGB1-TEAD4-KRT19* gene signature is significantly associated with poor overall survival and early recurrence.

### A Prognostic value of *ITGA2-ITGB1-TEAD4-KRT19* gene signature

| Gene A   | Gene B | p-Value  | Log Odds Ratio | Association       |
|----------|--------|----------|----------------|-------------------|
| ITGA2    | ITGB1  | <0.001   | 2.098          | Tendency towards co-occurrence |
| ITGA2    | TEAD4  | <0.001   | 1.942          | Tendency towards co-occurrence |
| ITGB1    | TEAD4  | <0.001   | 2.046          | Tendency towards co-occurrence |
| ITGB1    | KRT19  | <0.001   | >3             | Tendency towards co-occurrence |
| TEAD4    | KRT19  | <0.001   | 2.682          | Tendency towards co-occurrence |
| ITGA2    | KRT19  | 0.001    | 2.242          | Tendency towards co-occurrence |

**B Overall survival Kaplan-Meier (n=370)**

Logrank test p-value: 0.0336

**Disease free survival Kaplan-Meier (n=319)**

Logrank test p-value: 4.696e-4
**Supplementary Figure S6.** HepG2 cells with stable knockdown of Laminin B1 (shLB-27, shLB-29) or Keratin 19 (shK19-44, shK19-73) or the non-target control (shNT) were subcutaneously injected into NSG mice (n=8). Tumor length and width were measured every second day and the volume was calculated by the formula (length x width$^2$) x ½.

![Graph showing tumor volume vs. days for different knockdown groups.]

**Supplementary Figure S7.** Proliferation curves were determined for HepG2 and SNU423 cells with stable knockdown of Laminin B1 (shLB-27, shLB-29) or Keratin 19 (shK19-44, shK19-73) or the non-target control (shNT). 2 x 10$^5$ cells were cultured on a 6 well plate and cell numbers were counted every 24 hours with a CASY® cell counter.

![Graphs showing cell number vs. days for HepG2 and SNU423 cells with different knockdowns.]

**References:**

1. Govaere O, Komuta M, Berkers J, Spee B, Janssen C, de Luca F et al. Keratin 19: a key role player in the invasion of human hepatocellular carcinomas. Gut 2014; 63: 674-685.