Impact of NM23-M1 "knock-out" on metastatic potential of hepatocellular carcinoma: a transgenic approach
NM23-M1 "knock-out" mice (without NDPK A)

Experimental models of hepatocarcinogenesis

– Chemical model (Diethylnitrosamine, DEN) :
  single IP injection in 15-days-old mice (10µg/g body weight).

– Double transgenic mice : ASV/NM23-M1−/−
  breeding of NM23-M1 "knock-out" mice with ASV mice expressing specifically in the liver the SV40 large T antigen under the control of antithrombin III promoter and developping hepatocellular carcinoma (P. Briand).
Primary tumor development in DEN-treated and ASV mice

No difference in tumor primary formation between NM23-M1+/+ and NM23-M1−/− mice.
Immunohistochemical analysis with Hep-Par 1 antibody

Hep-Par 1: specific anti-hepatocyte antibody

Only the lung nodules of ASV mice are hepatocarcinoma metastases.
Metastatic dissemination in ASV mice

Highly significant increase in the metastatic incidence in ASV/NM23-M1−/− mice.
NM23 silencing and consequences in epithelial cancer cell models
**NM23-H1 silencing and cell scattering (1)**

**HepG2 cells**

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<th>48h</th>
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NM23-H1 silencing and cell scattering (2)

NM23-H1 silenced cells show a random spatial distribution
NM23-H1 silencing and cell scattering (3)

PLC/PRF/5 cells

HCT8/S11 cells

NM23-H1 silenced cells are scattered and show cellular extensions
NM23-H1 silencing and intercellular adhesion

HepG2 cells

**fast aggregation assay**

![Graph showing particle diameter distribution for HepG2 cells at different time points.]

- 1. HepG2, t=0 min
- 2. HepG2-Si1, t=30 min
- 3. HepG2-C, t=30 min
- 4. HepG2, t=30min

**slow aggregation assay**

![Images showing HepG2 cells at different conditions.]

HepG2 – C  
HepG2 – Si1  
HepG2 – Si2
NM23-H1 silencing and intercellular adhesion

**HCT8/S11 cells**

**fast aggregation assay**

Inhibition of intercellular adhesion by NM23-H1 silencing

**slow aggregation assay**

Inhibition of intercellular adhesion by NM23-H1 silencing

**Graph:**
- **1.** HCT8/S11, t= 0 min
- **2.** HCT8/S11-Si1, t= 30 min
- **3.** HCT8/S11-C, t= 30 min
- **4.** HCT8/S11, t= 30 min

**Images:**
- HCT8/S11 – C
- HCT8/S11 – Si1
- HCT8/S11 – Si2
NM23-H1 silencing and adherens junctions

NM23-H1 silencing induces the disruption of adherens junction complexes
NM23-H1 silencing induces β-catenin-TCF/LEF-1 transactivation through a GSK-3β-independent mechanism.
NM23-H1 silencing and cell motility

HepG2 cells

NM23-H1 silenced cells exhibit higher motility

NM23-H1 silenced cells exhibit higher motility
NM23-H1 silencing and cell motility

HCT8/S11 cells

NM23-H1 silencing induces directional migration
NM23-H1 silencing and actin cytoskeleton

Fluorescence microscopy

Scanning electron microscopy

NM23-H1 silencing induces filopodia-like structures and Rac1 activation
NM23-H1 silencing and invasion

- NM23-H1 silencing induces invasion in matrigel and collagen I.
- Conditioned media from control and silenced cells does not promote invasion.
NM23-H2 silencing and invasion

**Native collagen I**

NM23-H2 silencing does not induce invasion in collagen I.
The NDPK intrinsic activity of NM23-H1 only represents a minor fraction of the global cellular NDPK activity.
NM23-H1 silencing, invasion and signaling

HepG2 cells

HCT8/S11 cells

Involvement of MAPK, PI3K, Rho/ROCK, and Src in invasion induced by NM23-H1 silencing
Hyperactivation of MAPK and PI3K/Akt/S6K induced by NM23-H1 silencing
NM23-H1 silencing and invadopodia (1)
Depletion of NM23-H1 stimulates matrix degradation, invadopodia formation and invasion in a MT1-MMP-dependent manner.
NM23-H1 silencing and invasion in other studies

Invasion induced by NM23-H1 silencing
- in melanoma cell lines (Bakalian et al, 2007)
- in lung cancer cell lines (Ma et al, 2008)
- in trophoblast cells (Xie et al, 2010)

Microarray analysis: MMP-1 and MMP-2 upregulated genes, E-cadherin downregulated gene (Ma et al, 2008)

NM23-H1 silencing induced increased levels of activated FGF receptor (Hsu et al, 2006)
NM23-H1 silencing and proliferation

Flow cytometry

Soft agar assay

No effect on anchorage-dependent and-independent growth
NM23-H1 in hepatocellular carcinoma (1)
NM23-H1 in hepatocellular carcinoma (2)
NM23-H1 in colon carcinoma (1)
NM23-H1 in colon carcinoma (2)

Loss or reduction of NM23-H1 at the invasive front of clinical tumors in comparison to the central body of the tumor
Phénotype éphithélial (NM23-H1 >0)

Cohésion et polarité éphithéliale

Déstabilisation des jonctions adhérentes (E-cadhérine). Translocation nucléaire ($\beta$-caténine)

Phénotype mésenchymateux (NM23-H1 <0)

Dispersion cellulaire

Perte de NM23-H1 au front invasif de tumeurs primaires

MEK

ERK

MT1-MMP

Akt

LEF-TCF (Wnt)

β-caténine

Src

Rac1-GTP

Tiam-1

MEK

PI3K

JNK

p38

TEM partielle

Motilité

INVASION

Collagène type I

Matrigel

Gélatine

Invadopodes (MT1-MMP)