Models to study cancer stem cells in gastric carcinoma

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Gastric carcinoma

Epidemiological datas :

- 2\textsuperscript{e} cause of mortality by cancer in the world
- > 600 000 deaths each year
- 5\textsuperscript{e} rank in France, 6 500 new cases/year
- age : 70 years old
Gastric carcinoma

Epidemiological datas :

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A cancer of bad prognosis : 

- survival rate after 5 years < 20%
- surgery, conventional chemotherapy, no specific therapy (excepted for the 20% of Her2+ cases)
Factors associated to gastric carcinoma

- Environnemental factors
- Host factors
- Bacterial factors
Factors associated to gastric carcinoma

- Environmental factors
  - tobacco,
  - diet (salt, brine food, vit. deficiency, red meat / nitrosamines)

- Host factors

- Bacterial factors

Gastric carcinoma
Factors associated to gastric carcinoma

- Environmental factors
- Host factors
  - Genetic susceptibility:
    - Polymorphism of IL-1β, IL-1RN, TNFα-R, IL-8, IL-10
    - cdh1 mutations
- Bacterial factors
Factors associated to gastric carcinoma

Environnmental factors

Host factors

Gastric carcinoma

Bacterial factors

*H. pylori* infection associated to >93% of non-cardia gastric carcinoma cases (Gonzalez, Mégraud et al. Ann. Oncol. 2012)
Distal gastric adenocarcinoma linked to *H. pylori*

Gonzalez *et al.* Ann. Oncol. 2012: > 93% associated to *H. pylori* infection
Carcinogenicity of \textit{H. pylori}

- Gram- bacteria, microaerophilic, motile
- 1994: class I carcinogen by the WHO
- Main pathogenic factor associated to gastric carcinoma:
  - \textbf{oncoprotein CagA}: cytotoxin encoded by the \textit{cag} pathogenicity island
The type IV secretion system and effects of CagA on host cell

From Backert et al, Cell Microbiology 2008
The type IV secretion system and effects of CagA on host cell

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From Backert et al, Cell Microbiology 2008
Carcinogenicity of CagA

- In mongolian gerbils: infection with a CagA+ *H. pylori* strain induces the development of gastric carcinoma after 7 months

- Expression of CagA in a transgenic mouse model: gastric hyperplasia & adenocarcinomas

From Ohnishi *et al*, PNAS 2007;105:1003-8

From Franco *et al*, PNAS 2005;102:10646-51
Cancer Stem Cells

- CSC = sub-population within the tumor with:
  - self-renewal properties
  - asymmetrical division and differentiation properties
  - expression of specific markers
  - capacity to initiate a new tumor
CD44, a marker of CSC in gastric cancer cell lines

Takaishi et al., Stem Cell 2009:

- Tumorsphere assay
- Xenograft in NOD/SCID mice

CD44+

CD44-

Orthotopic injection

Subcutaneous injection

MKN-45

N-87
To confirm the existence of CSC in primary human gastric adenocarcinoma
Strategy to identify gastric CSC markers

Consentent patients
n = 49

Bulk xenograft
n = 39

3-5 months

NSG

Secondary tumors
n = 8
Strategy to identify gastric CSC markers

Consentent patients
n = 49

Bulk xenograft
n = 39

NSG

Secondary tumors
n = 8

3-5 months

Cellular dissociation

Analysis of the expression of putative CSC cell surface markers (11)

Cell sorting / FACS

Study of tumorigenic properties

in vitro

in vivo
Expression of putative CSC markers in gastric cell lines and primary tumors

- markers expressed at a lower level in primary tumor cells vs. cell lines
- CD44 expressed in ~20% of cells from primary tumors
Cell sorting of sub-populations of tumor cells on the expression of markers of interest

- Cell sorting / FACS on cancer cells dissociated from tumors:

  * ESA-FITC
  * CD133-PE
  * ESA-FITC
  * CD133-PE

  * CD44-APC

  FACS cell sorting

  Study of tumorigenic properties

  * in vitro
  * in vivo
Study of tumorigenic properties of sorted cancer cells expressing or not CD44

- Tumorsphere assay:

- Xenograft in NSG mice (ELDA):

<table>
<thead>
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<th>Table 2: Gastric cancer-initiating cell frequencies for the marker CD44</th>
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→ CD44+ cells form tumorspheres *in vitro* & tumors *in vivo*

→ frequency of CD44+ tumor initiating cells: 0.1 – 2%

→ CD44: marker of CSC in primary human gastric adenocarcinoma

Nguyen *et al.*, in preparation
1 / Gastric CSC exist

2 / Origin of gastric CSC?

3 / How do these cells transform in response to H. pylori?
Cellular origin of gastric CSC?

- CSC in gastric carcinoma may originate:
  - 1/ from local epithelial stem cells:
Cellular origin of gastric CSC?

- CSC in gastric carcinoma may originate:
  - 1/ from local epithelial stem cells:
    - Villin, antrum (Qiao, Gastroenterology 2007)
    - Lgr5, pylorus & cardia (Barker et al. Cell Stem Cell 2010)
    - Dcamkl1, isthmus in corpus (Okumura et al. Gastroenterology 2010)
    - CD44, isthmus in corpus (Khurana et al. JBC 2013)
    - TFF2 (Quante, Gastroenterology 2010)
    - Mist1 (Nam et al. Gastroenterology 2010)
    - Troy, Wnt signature (Lgr5 & CD44) (Stange et al. Cell 2013)

From Goldenring et al., Gastroenterology 2010
Cellular origin of gastric CSC?

CSC in gastric carcinoma may originate:

1. from local epithelial stem cells
2. from stem cells derived from the bone marrow (BMDC):
   - mouse model of gastric carcinogenesis induced by infection with *H. felis* (Houghton *et al.*, Science 2004)
Cellular origin of gastric CSC?

- CSC in gastric carcinoma may originate:

  → 1/ from local epithelial stem cells?

  → 2/ from stem cells derived from the bone marrow (BMDC):

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Is it also true in gastric carcinoma associated to the human pathogen *H. pylori*?
Mouse model to study BMDC involvement in *H. pylori*-induced gastric carcinogenesis

900 cGy

C57Bl/6

BM transplantation

GFP+ C57Bl/6

chimera (GFP+ and chr Y+ BMDC)
Mouse model to study BMDC involvement in 
*H. pylori*-induced gastric carcinogenesis

- 900 cGy
- C57Bl/6
- BM transplantation
- GFP+ C57Bl/6
- Chimera (GFP+ and chr Y+ BMDC)
- H. felis
- H. pylori
- 15, 35, 55, 75 weeks ⇒ study of BMDC recruitment in gastric neoplastic lesions

Varon et al., Gastroenterology 2012
Mouse model to study BMDC involvement in *H. pylori*-induced gastric carcinogenesis

15 weeks: Inflammation

35 weeks: Hyperplasia / Oxyntic atrophy
Mucous metaplasia (TFF2+)

55 weeks: Pseudo-intestinal metaplasia
(TFF3+, alcaline phosphatase+)

75 weeks: Dysplasia & Gastrointestinal
Intra-epithelial neoplasia (GIN) :
BMDC within neoplastic lesions induced by *H. pylori*

- Detection of BMDC by GFP immunohistochemistry on gastric mucosa:

  - Chimera **after 1 year**:
    - *H. pylori*
    - + *H. pylori*

  [Images of tissue samples with BMDC detection]

  
  **GFP+ dysplasia (GIN) : 22%**

Varon *et al.*, Gastroenterology 2012
*H. pylori*-induced neoplastic lesions in mice are composed of CD44+ cells

- IHC CD44 on stomach of mice infected with *H. pylori*:

  - normal
  - metaplasia
  - GIN

Varon et al., Gastroenterology 2012
1 / Gastric CSC exist

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3 / How do these cells transform in response to *H. pylori*?
In vitro, *H. pylori* infection alters epithelial differentiation

Altered differentiation:

- intestinal markers (MUC2 and Cdx1)
  
  Murata-Kamiya *et al.*, Oncogene 2007  
  Zhu *et al.*, PLoS ONE 2012

- epithelial to mesenchymal transition

  Bagnoli *et al.*, PNAS 2005  
  Yin *et al.*, Gut 2010  
  Baud *et al.*, PLoS ONE 2013
The epithelial to mesenchymal transition (EMT)

- development
- inflammation, healing
- tumoral progression (metastasis)

EMT induced in mammary epithelial cells generates cells with breast CSC properties

- Immortalized mammary epithelial cells
- Snail or Twist overexpression
- Induction of EMT

Appearance of cells with the phenotype and properties of breast CSC
- CD44<sup>high</sup>/CD24<sup>low</sup>
- Migration
- Self-renewal
- Tumorspheres in vitro
- Tumors in vivo

EMT generates cells with CSC properties

Morel et al., PloSONE 2008
Mani et al., Cell 2008
Can *H. pylori*, via an EMT, generate cells with CSC properties?
Cells harboring a mesenchymal phenotype in response to *H. pylori* infection are CD44\textsuperscript{high}

*H. pylori* via CagA induces:
- a mesenchymal phenotype
- molecular markers of the EMT
- cells harboring the mesenchymal phenotype overexpress CD44

Bessède *et al.*, Oncogene 2013
CD44\textsuperscript{high} cells induced by \textit{H. pylori}/CagA harbor mesenchymal features and CSC properties

7.13-CD44\textsuperscript{high}

7.13-CD44\textsuperscript{low}

Fold change of mRNA level

Fold change in spheroids formation

Fold change of migration

Tumors volume in mice

\textsuperscript{*}

\textsuperscript{b}

Bessède \textit{et al.}, Oncogene 2013

\textbf{CD44}\textsuperscript{high} - \textbf{CD44}\textsuperscript{low}
CD44 and EMT markers are overexpressed in *H. pylori*-infected human gastric mucosa and in carcinoma

Bessède *et al.*, Oncogene 2013
Cellular and molecular determinants of *H. pylori*-induced gastric carcinogenesis

1. Inflammation & epithelial damages
2. Chemotactism
3. BMDC recruitment
4. Homing and differentiation
5. Altered differentiation / metaplasia
6. Dysplasia
7. Emergence of CD44<sup>high</sup> CSC and carcinoma

Composed at 22% of BMDC
Composed of CD44<sup>+</sup> cells with CSC properties
Transformation

From Bessède *et al.*, Oncogene review 2014

Ferrand *et al.*, PLoS ONE 2011a
Ferrand *et al.*, PLoS ONE 2011b
Varon *et al.*, Gastroenterology 2012
Baud *et al.*, PLoS ONE 2013
Bessède *et al.*, Oncogene 2013
Nguyen *et al.*, in preparation
Collaborations:
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- C. Staedel (INSERM U869)
- F. Mazurier (INSERM U1035)
- I. Soubeyran, S. Evrard (Institut Bergonié)
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- L. Wittkop (USMR, ISPED)
- A. Schmidt-Alliana (INSERM U576, Nice)
- D. Noel (INSERM U844, Montpellier)
- M. Hatakeyama (University of Tokyo, Japon)

Platforms:
- Cytometry (V. Pitard, S. Gonzales)
- Animal facilities A2 (B. Rousseau) & transgenic (P. Costet)
- Histology (N. Senant)
- Vectorologie (V. Guyonnet-Dupeyrat)

Financial supports:
CagA is responsible of the induction of mesenchymal markers and phenotype, and of CD44 overexpression.

Bessède et al., Oncogene 2013
ongoing project: constitution of TMA of gastric adenocarcinoma

→ to confirm the expression of CD44 and markers of EMT and CSC in human tumors

250 cases: distal adenocarcinoma + distant non-cancerous mucosa

PET
Selection of areas of interest

sampling (1 mm Ø)

Paraffine embedding

IHC on section

analyses


G. Belleannée CHU Bordeaux
P. Dubus, A. Giese EA2406
L. Chambonnier

→ Markers of pre-neoplastic lesions? Predictive of severity, prognosis?
Cellular and molecular determinants of *H. pylori*-induced gastric carcinogenesis

- Mixed Dysplasia
- BMDC
- CD44$^{\text{high}}$ cells with CSC properties
- Local epithelial stem cells
- chemotactism
- BMDC recruitment
- Inflammation & epithelial damages
- Altered differentiation
- Metaplasia
- EMT
- Transformation
- CD44$^{\text{high}}$ cells with CSC properties
- adenocarcinoma

References:
- Ferrand *et al.*, PLoS ONE 2011a
- Ferrand *et al.*, PLoS ONE 2011b
- Varon *et al.*, Gastroenterology 2012
- Baud *et al.*, PLoS ONE 2013
- Bessède *et al.*, Oncogene 2013
- Bessède *et al.*, Oncogene 2014
Some strains of *H. pylori* stimulate MSC migration

No association with the *cagPAI* or *VacA* status of strains

Associated to the capacity of strains to induce epithelial cells apoptosis

Ferrand et al., PLoSOne 2011;6(12):e29007
$H.\text{pylori}$-induced MSC migration depends on NFκB activation and secretion of TNFα

\[\rightarrow\text{strain 7.13 « pro-migratory »}\]

\[\text{Strain 26695 « non-migratory »}\]

\[\text{Identification of chemokines involved in BM-MSC recruitment}\]

\[\text{Ferrand et al., PLoSOne 2011;6(12):e29007}\]
BM-MSC acquire an epithelial phenotype through cell fusion in vitro

BM-MSC eGFP
gastric epithelial cells DsRED (AGS)

→ 1.3% of fused cells after 8 days of coculture
→ loss of mesenchymal markers, gain of epithelial markers

Ferrand et al., PLoSOne 2011;6(5):e19569
Stem cells in adult tissues

- Self-renewal
- Asymmetrical division
- Differentiation in the different specialized cells of the tissue

From Goldenring et al., Gastroenterology 2010

From Eckfeldt et al., Nature Reviews 2005
Cancer stem cells

1994 : in AML (Lapidot et al. 1994; Bonnet & Dick 1997)

Since 2003, in solid tumors :

- Breast (Al-Hajj et al., 2003)
- Brain (Hemmati et al., 2003; Singh et al., 2003)
- Colon (O’Brien et al., 2007; Ricci-Vitiani et al., 2007)
- Pancreas (Hermann et al., 2007; Li et al., 2007)
- Prostate (Collins et al., 2005)
- Ovary (Bapat et al., 2005)
- Liver (Ma et al., 2007)
- Lung (Ho et al., 2007)
- Melanoma (Fang et al., 2005)
- Stomach in gastric cancer cell lines (Takaishi et al., 2009)