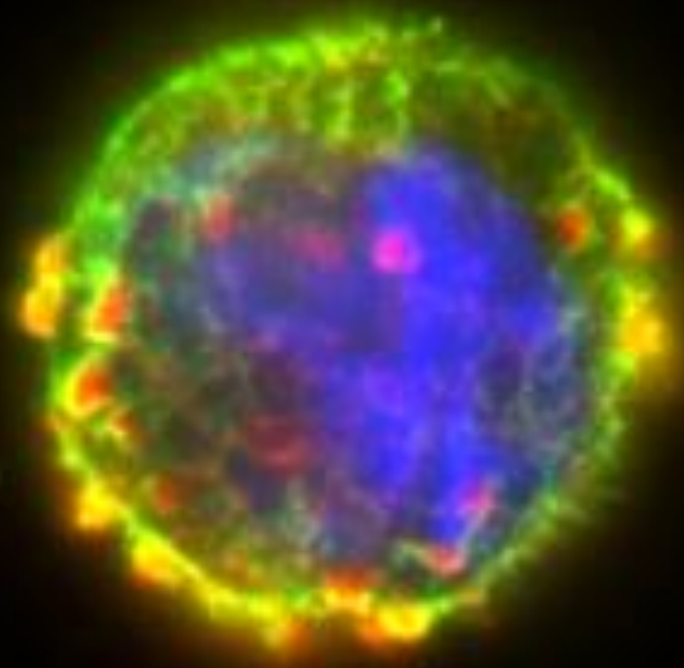
Models to study cancer stem cells in gastric carcinoma



Christine Varon, PhD, HDR, Associate Professor

INSERM U853 « *Helicobacter* infection, inflammation and cancer »
University of Bordeaux
Dir. : Francis Mégraud

BRIO BORDEAUX | Recherche Intégrée Oncologie

université de BORDEAUX

Inserm
Institut national de la santé et de la recherche médicale

Gastric carcinoma

Epidemiological datas :

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

Gastric carcinoma

Epidemiological datas :

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- > 600 000 deaths each year
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- age : 70 years old

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

Gastric carcinoma

Bacterial factors

Factors associated to gastric carcinoma

Environnemental factors

- tobacco,
- diet (salt, brine food, vit. deficiency, red meet / nitrosamines)

Host factors

Gastric carcinoma

Bacterial factors

Factors associated to gastric carcinoma

Environnemental factors

Host factors

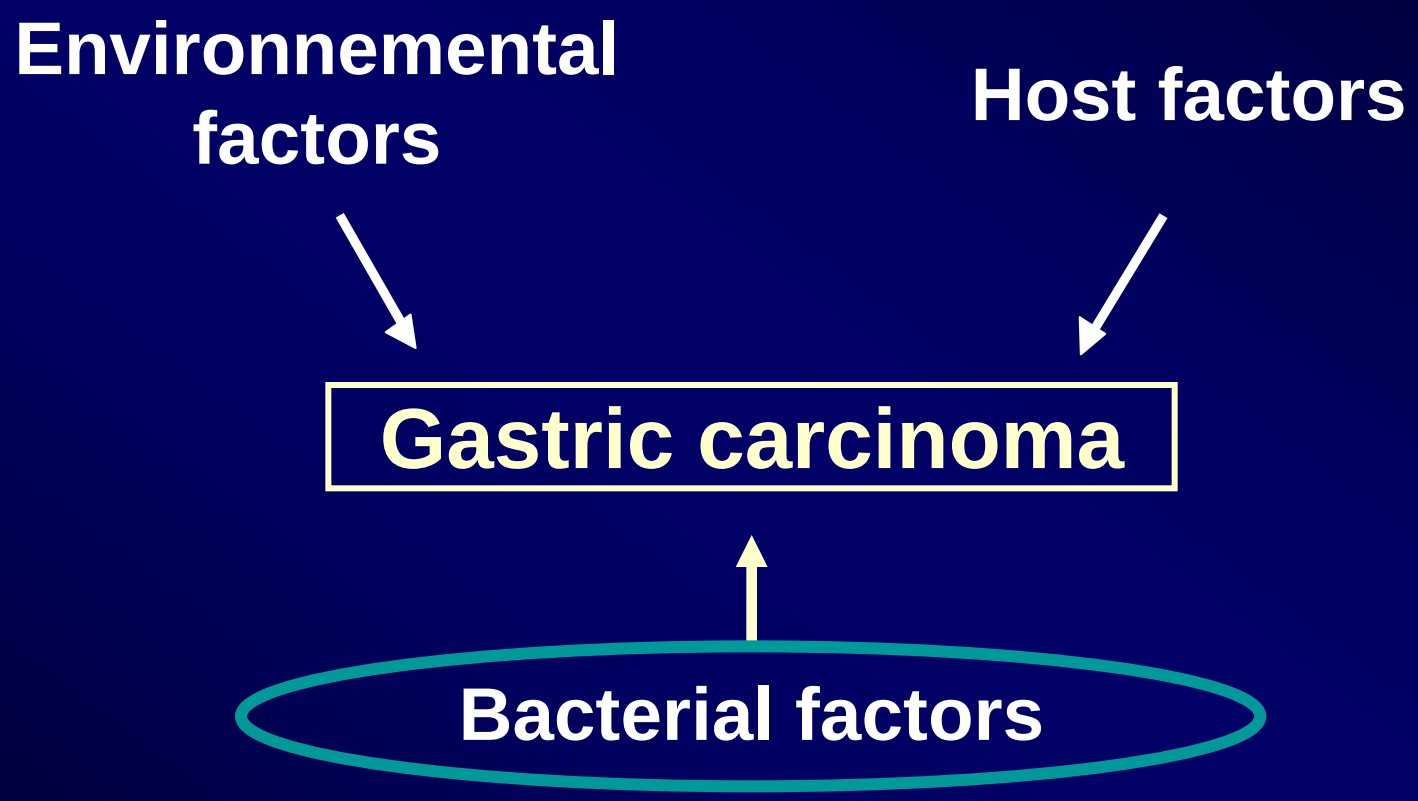
Gastric carcinoma

Bacterial factors

Genetic susceptibility :

- Polymorphism of IL-1β, IL-1RN, TNFα-R, IL-8, IL-10
- *cdh*1 mutations

Factors associated to gastric carcinoma

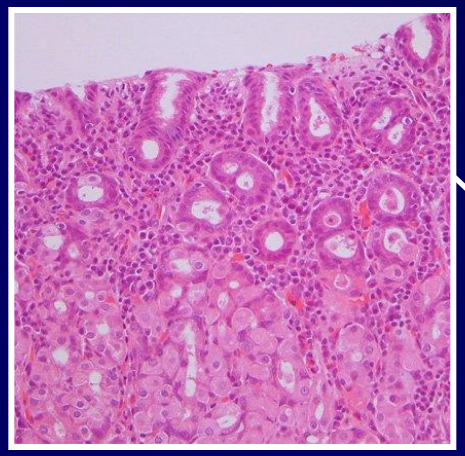


H. pylori **infection associated to >93% of non-cardia gastric carcinoma cases** **(Gonzalez, Mégraud *et al.* Ann. Oncol. 2012)**

Distal gastric adenocarcinoma linked to *H. pylori*

Gonzalez *et al*. Ann. Oncol. 2012 : > 93% associated to *H. pylori* infection

Chronic inflammation



Atrophy

↓

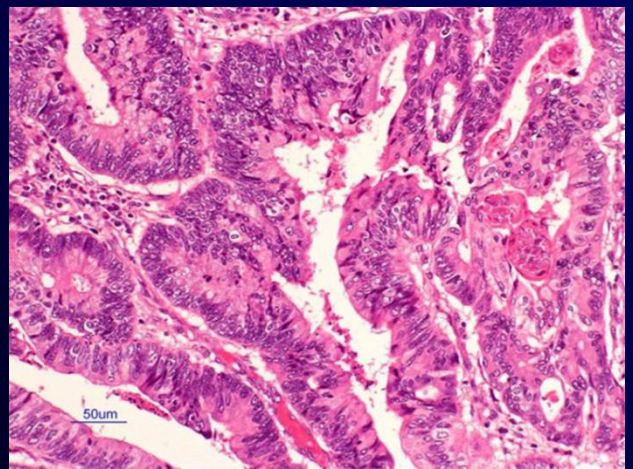
Intestinal metaplasia

↓

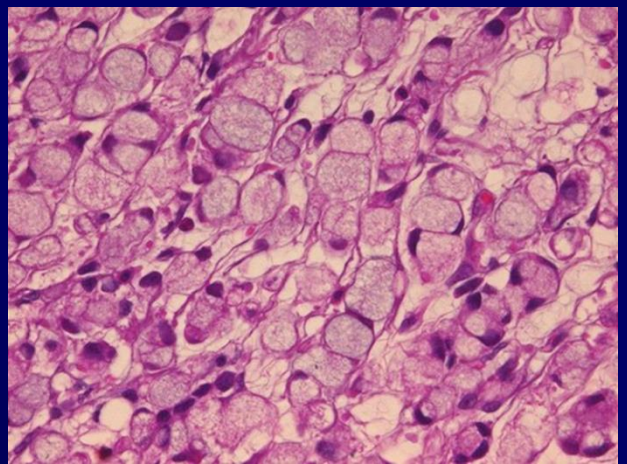
Dysplasia

↓

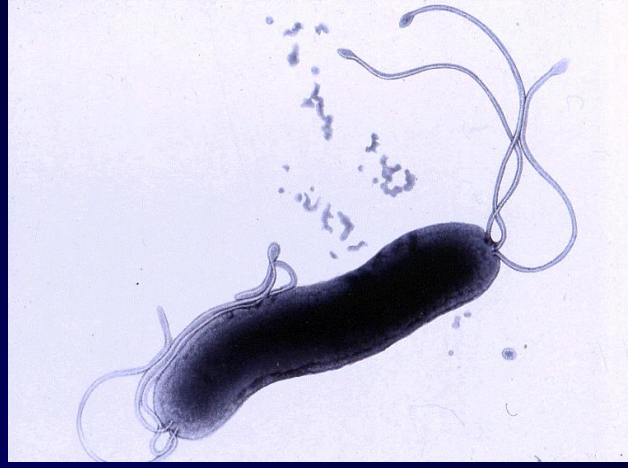
Adenocarcinoma of intestinal type



Adenocarcinoma of diffuse type



Carcinogenicity of *H. pylori*

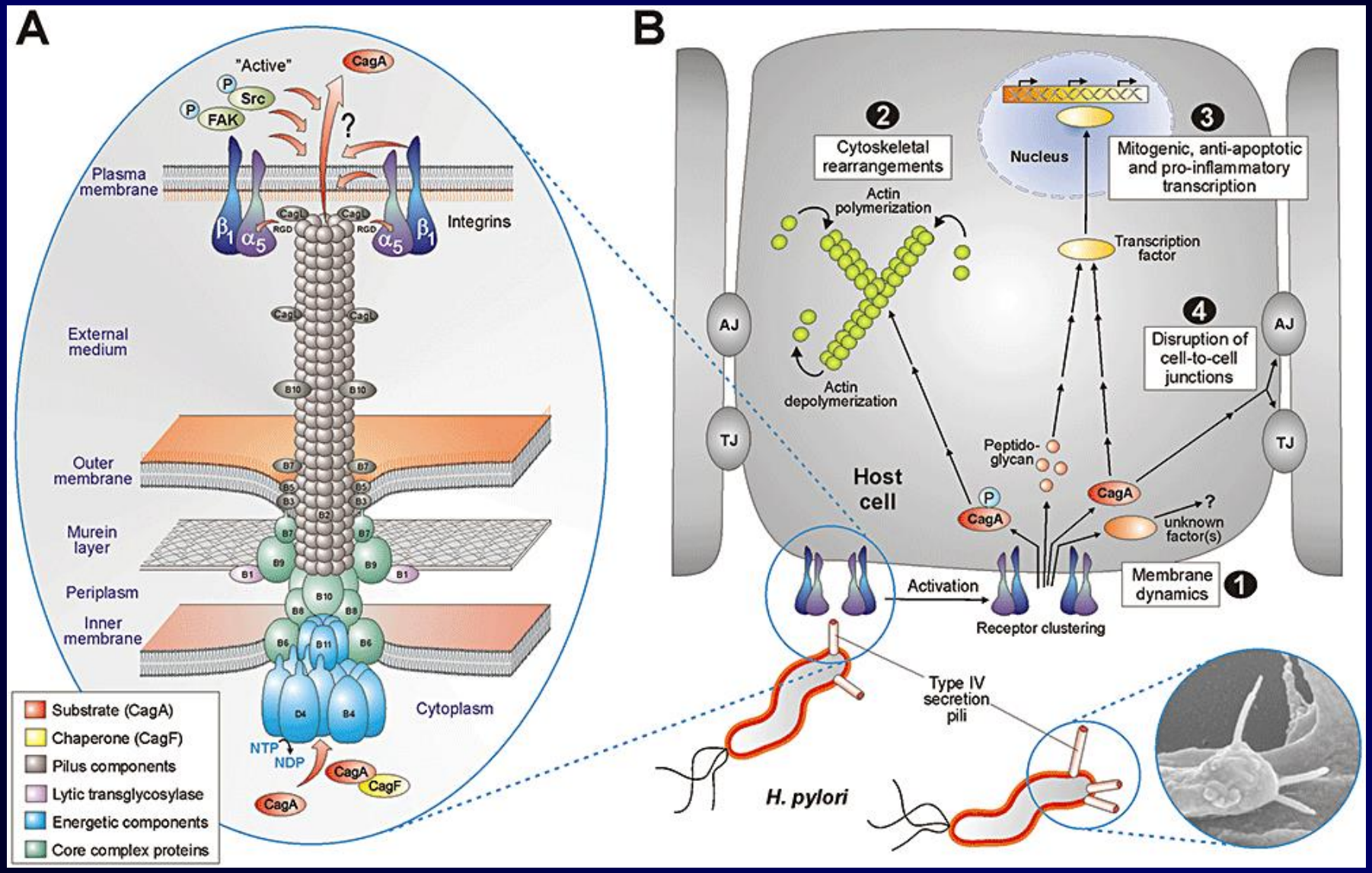


- **Gram- bacterium, microaerophilic, motile**
- **Isolated in 1982 (Warren & Marshall, Lancet 1984; Nobel Prize of Medecine 2005)**
- **1994 : class I carcinogen by the WHO**

- **Main pathogenic factor associated to gastric carcinoma :**

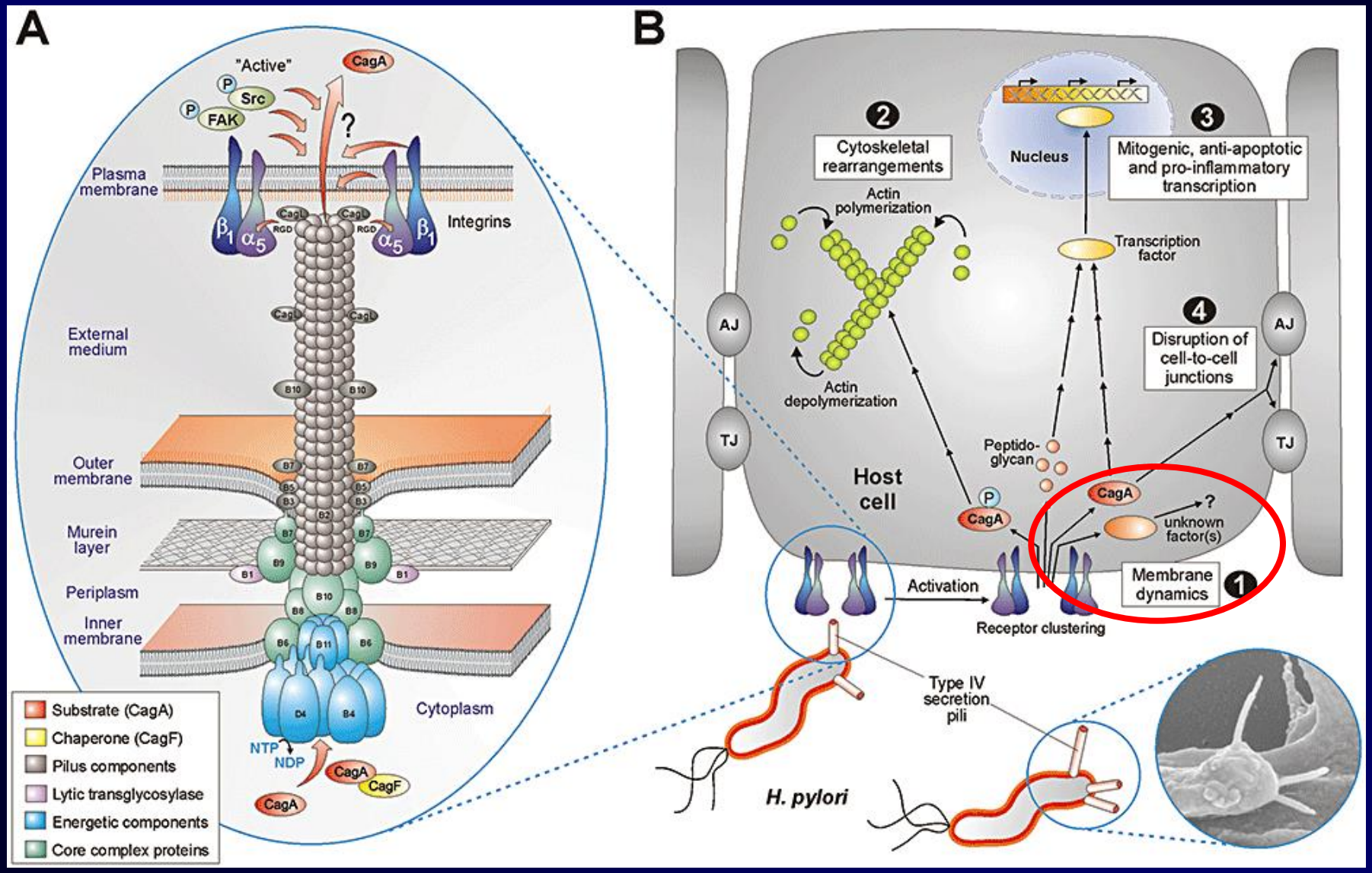
  - oncoprotein CagA : cytotoxin encoded by the *cag* pathogenicity island

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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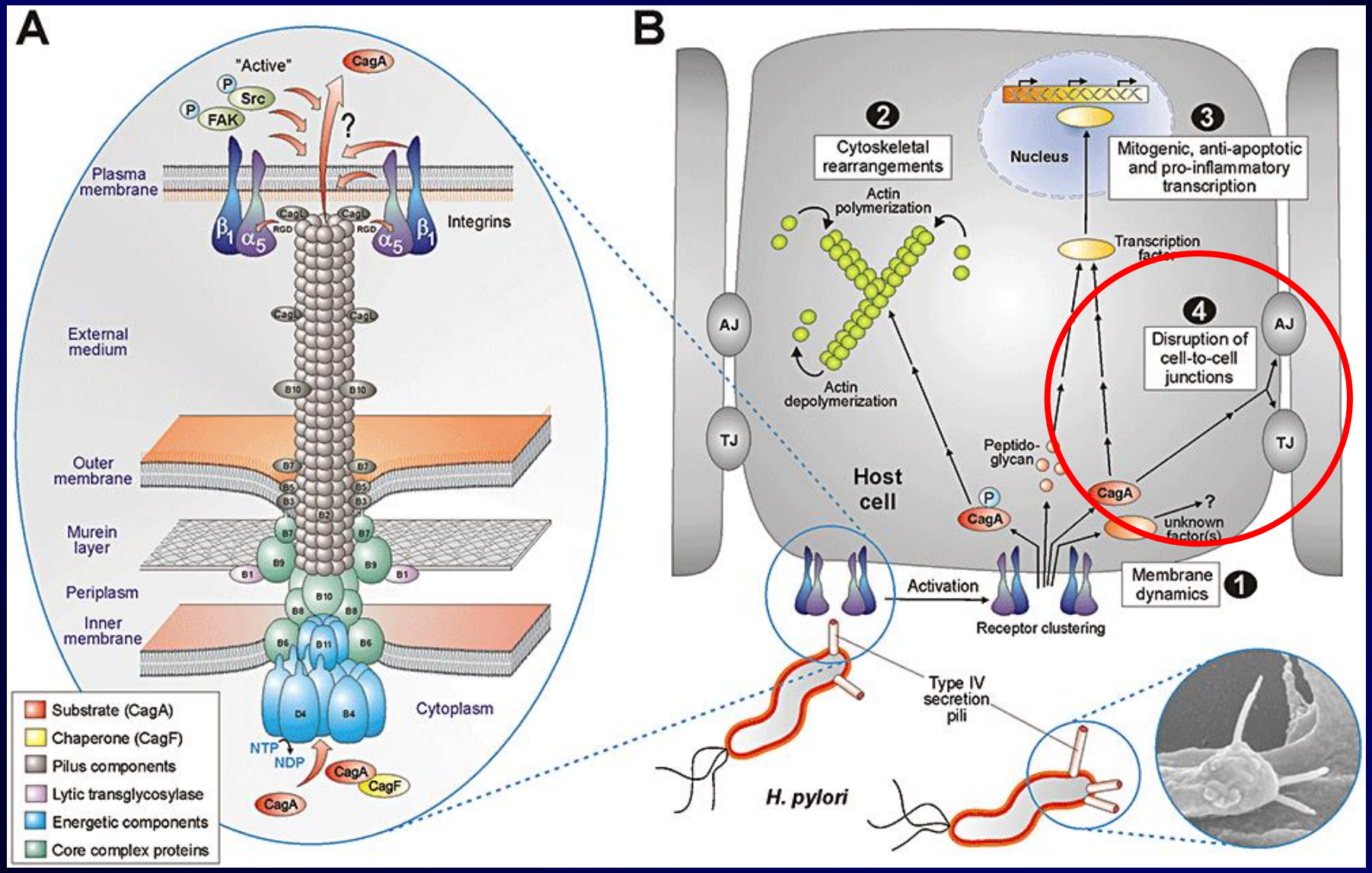

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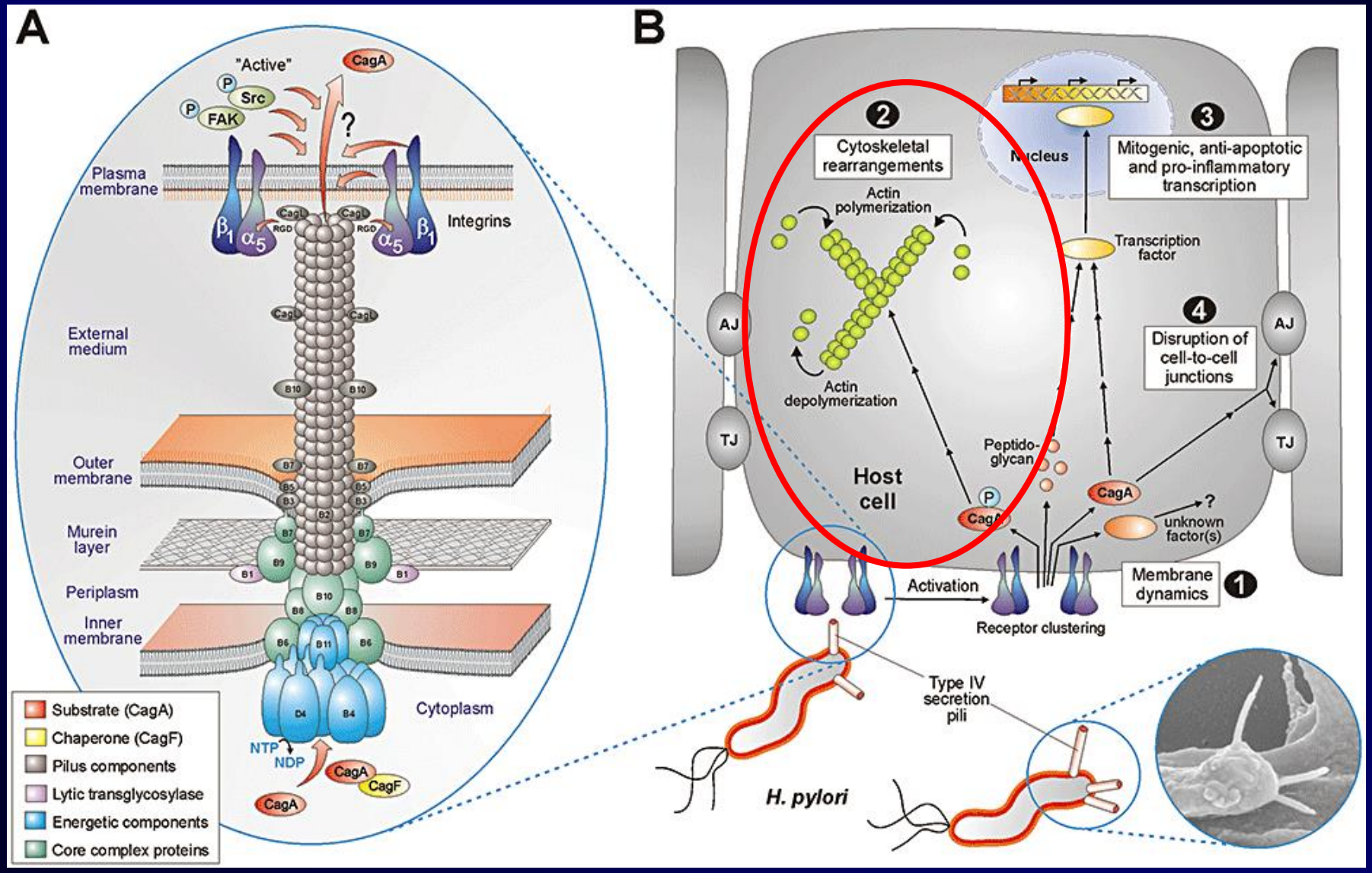

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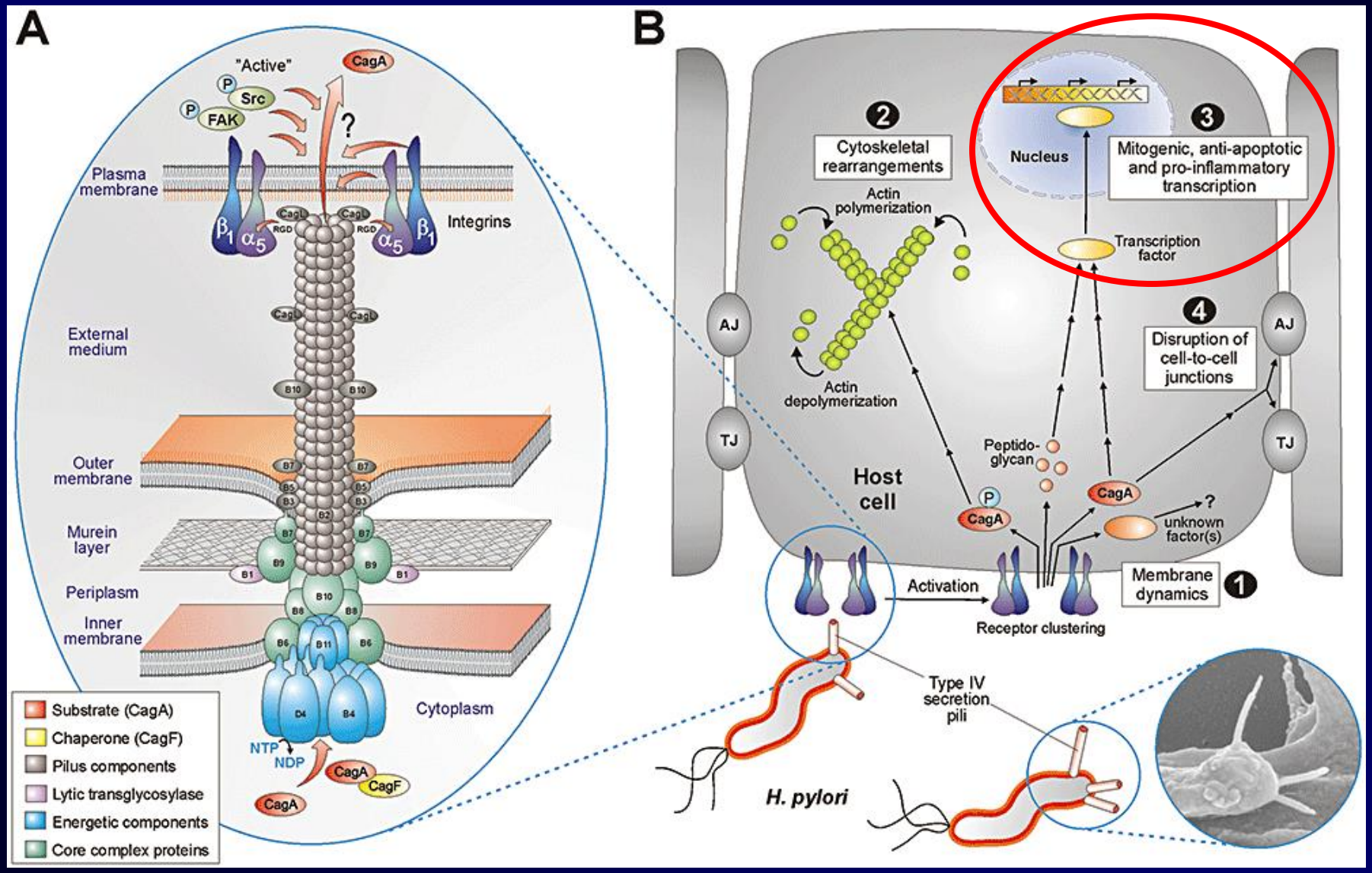
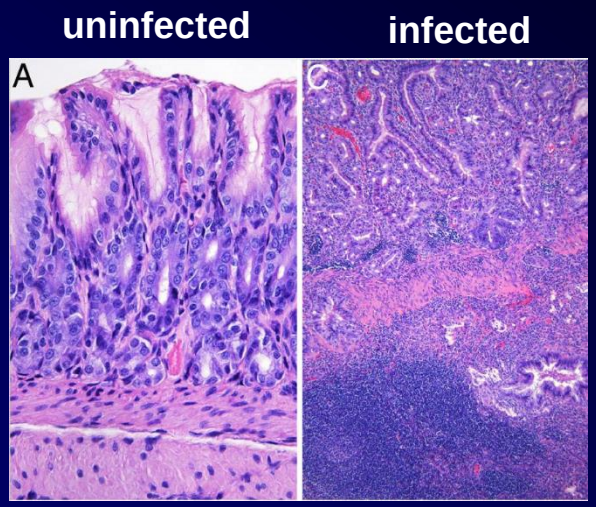


From Backert *et al*, Cell Microbiology 2008

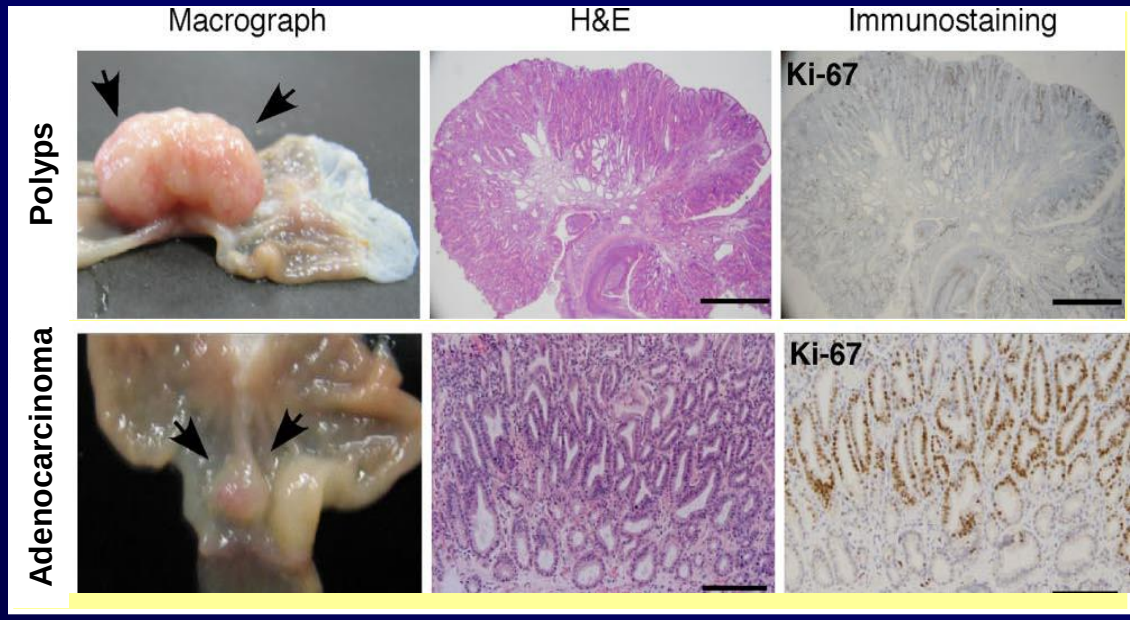
Carcinogenicity of CagA

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



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

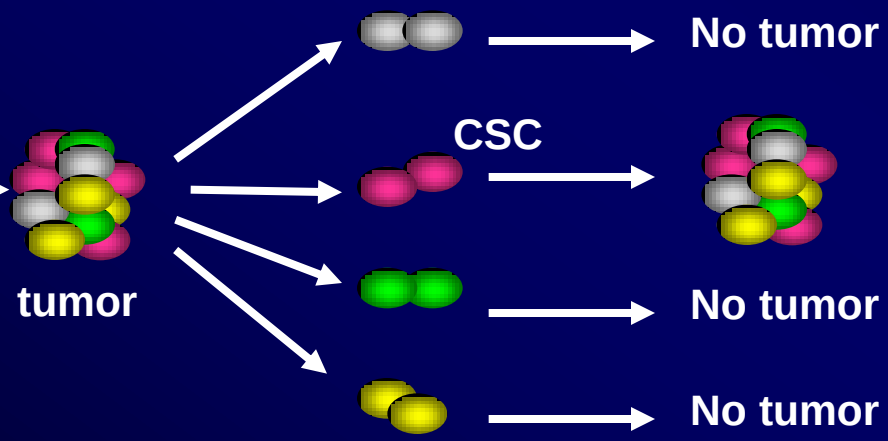
- Expression of CagA in a transgenic mouse model : gastric hyperplasia & adenocarcinomas



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

Cancer Stem Cells

- **CSC = sub-population within the tumor with :**
  - **self-renewal properties**
  - **asymetrical division and differentiation properties**
  - **expression of specific markers**
  - **capacity to initiate a new tumor**



Tumorsphere

Tumor after xenograft

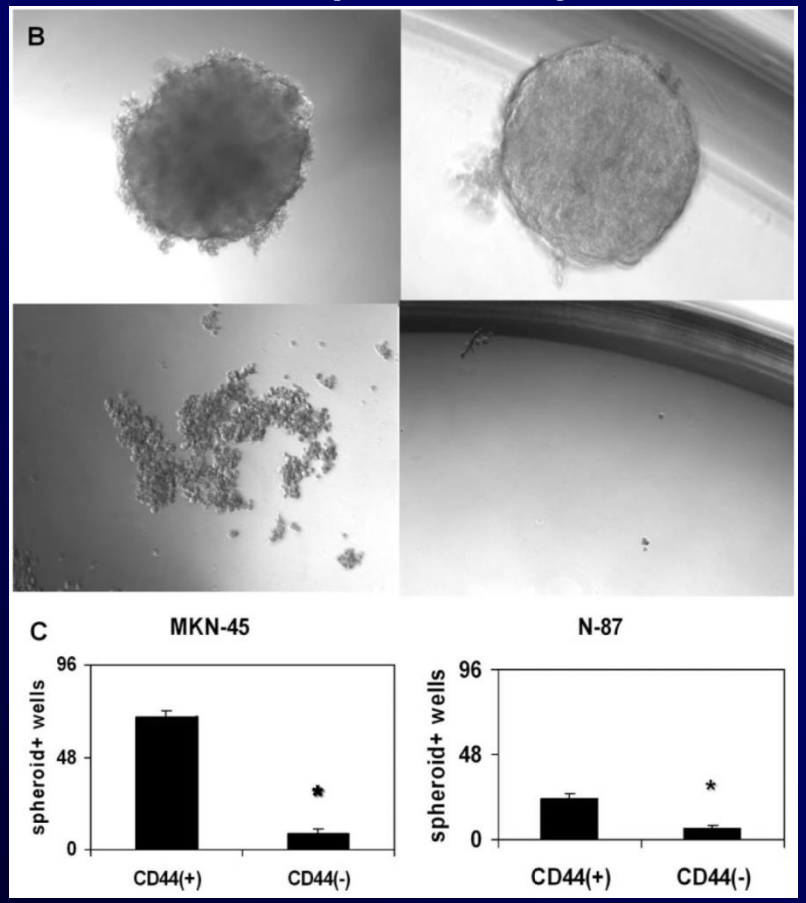
CD44, a marker of CSC in gastric cancer cell lines

Takaishi *et al*., Stem Cell 2009 :

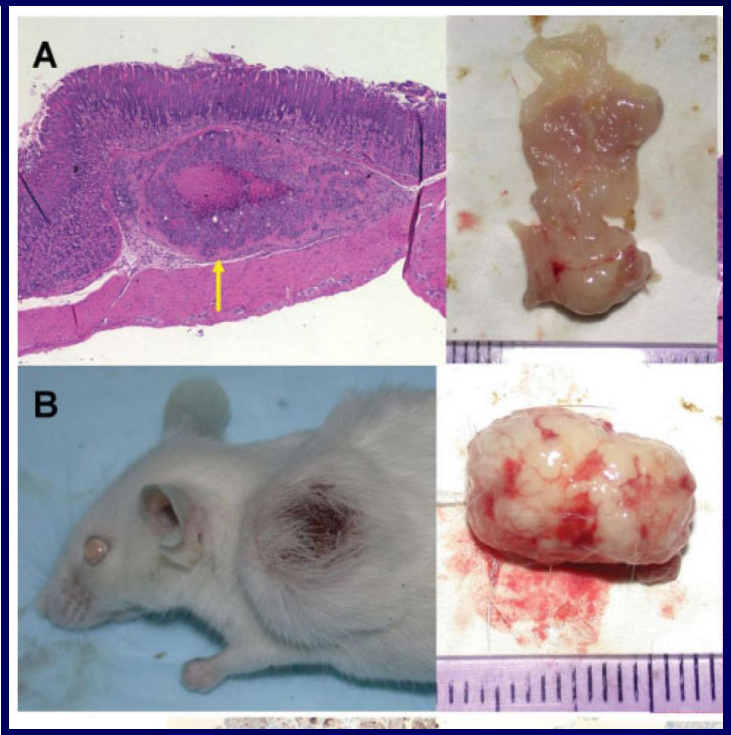
Tumorsphere assay

CD44+

CD44-



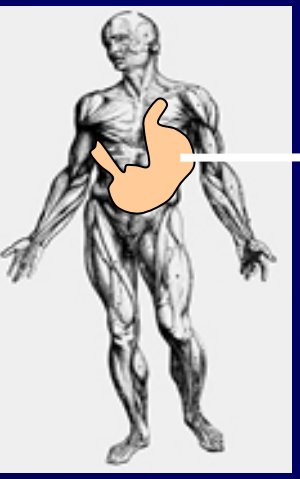
Xenograft in NOD/SCID mice



Orthotopic injection

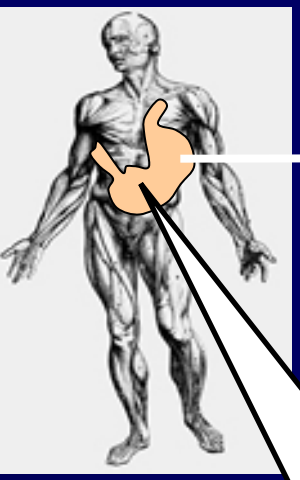
Subcutaneous injection

Aim



- To confirm the existence of CSC in <u>primary human gastric adenocarcinoma</u>

Strategy to identify gastric CSC markers



Consentent patients

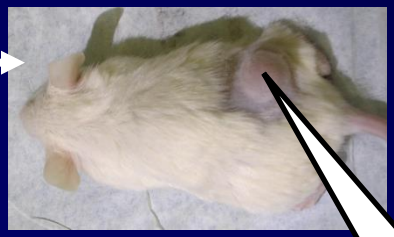
n = 49

Bulk xenograft

n = 39

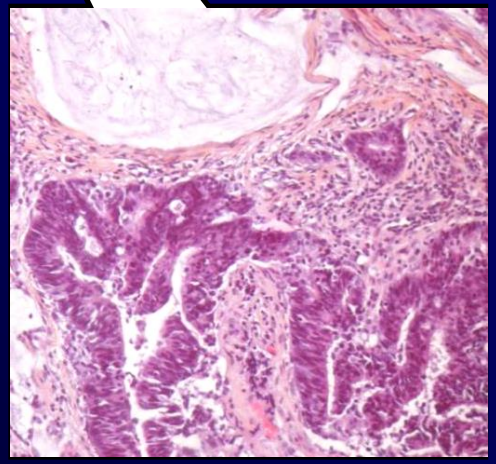
3-5 months

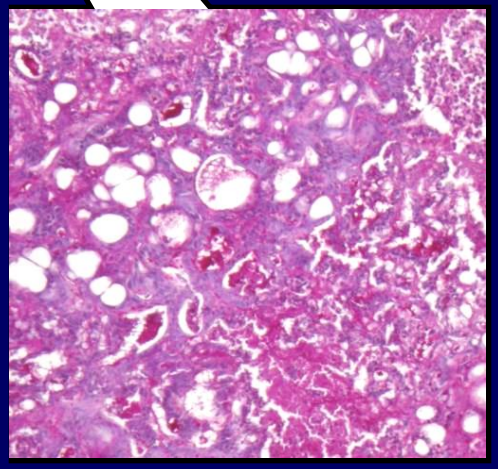
NSG



Secondary tumors

n = 8





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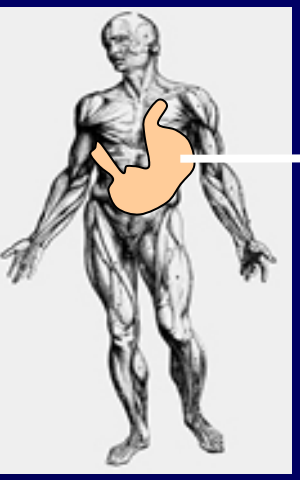

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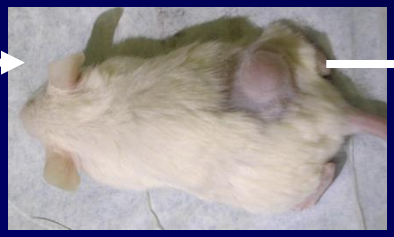


Secondary tumors

n = 8

Cellular dissociation

↓

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

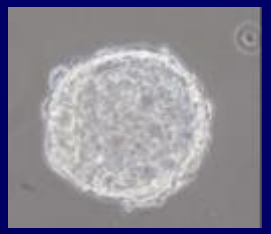
↓

Cell sorting / FACS

↓

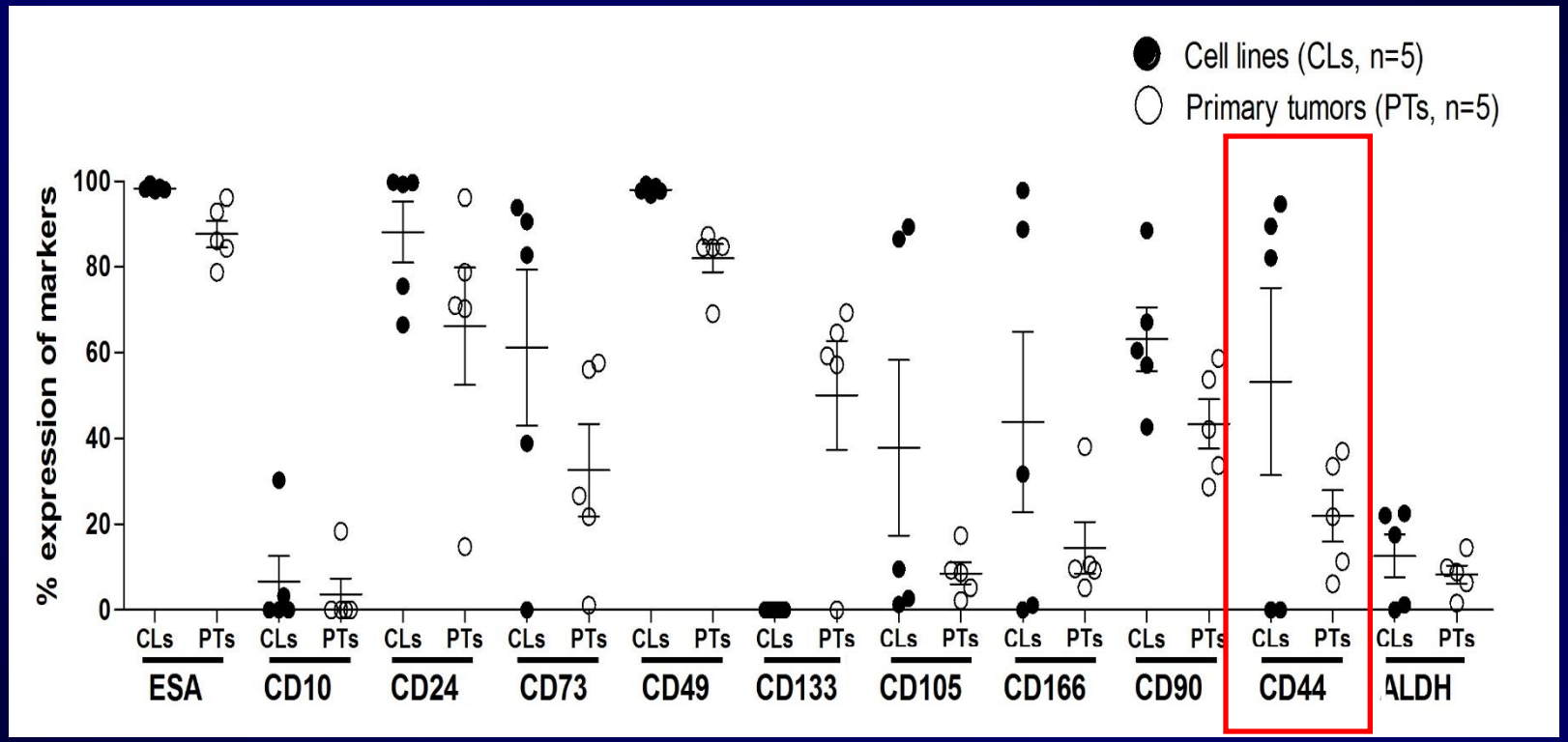
Study of tumorigenic properties

in vitro



in vivo

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

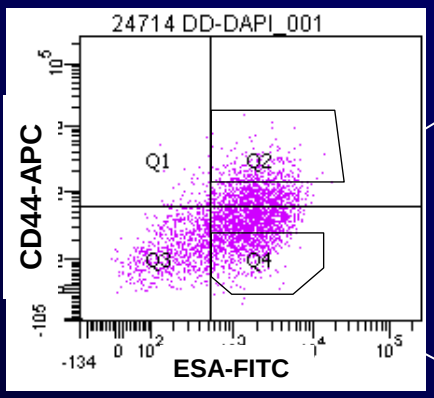


- markers expressed at a lower level in primary tumor cells vs. cell lines

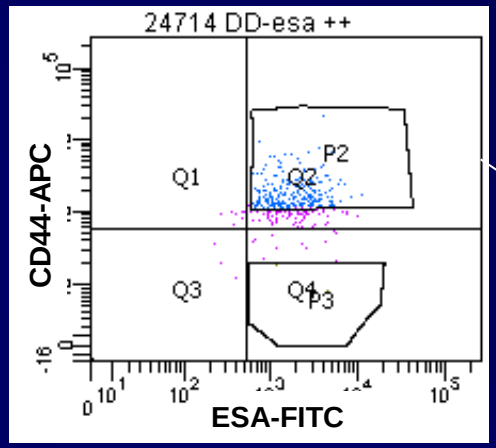
- CD44 expressed in ~20% of cells from primary tumors

Cell sorting of sub-populations of tumor cells on the expression of markers of interest

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

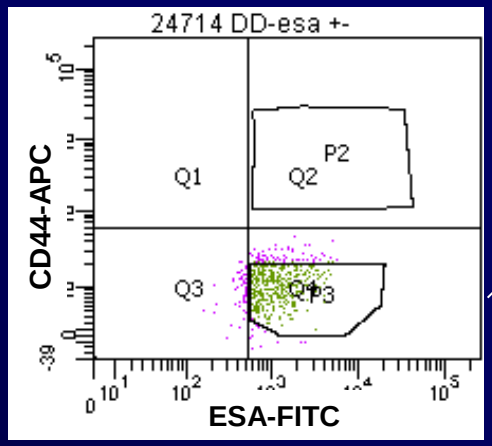


ESA+CD44+



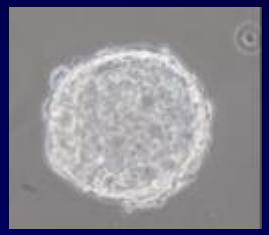
FACS cell sorting

ESA+CD44-

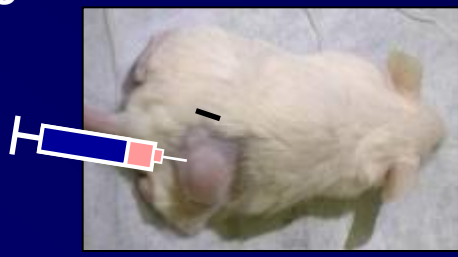


Study of tumorigenic properties

in vitro

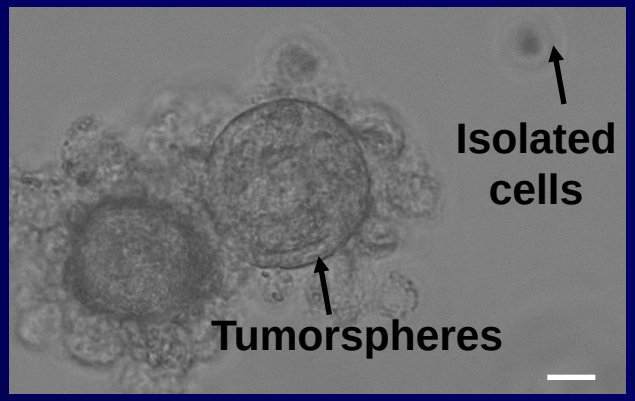


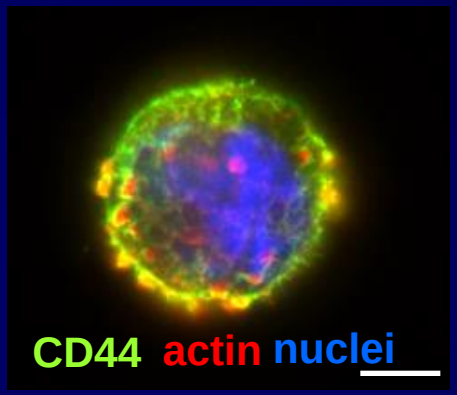
in vivo

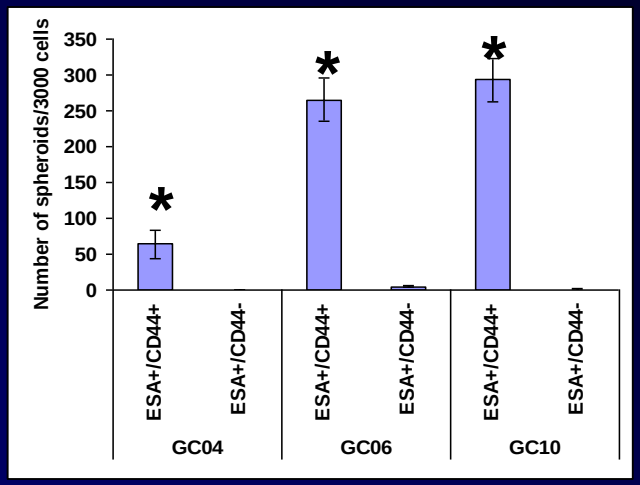


Study of tumorigenic properties of sorted-cancer cells expressing or not CD44

- **Tumorsphere assay:**







- **Xenograft in NSG mice (ELDA):**

Table 2: Gastric cancer-initiating cell frequencies for the marker CD44

CASE	Marker	Number of tumours/number of transplanted mice; number of cells transplanted cells: 10 000	3 000	1 000	300	100	100	Gastric cancer-initiating cell frequencies (95% Confidence intervals)		Test for difference in stem cell frequencies between CD44+ and CD44 negative cells
1	ESA+CD44+	8/8		9/10	6/10	2/5		1/352	(1/625-1/198)	*P*=0.0012
1	ESA+CD44-	4/4		4/8	1/8	0/5		1/1688	(1/3913-1/728)	
2	ESA+CD44+	5/5	5/5	8/15	4/15	2/10		1/1020	(1/1670-1/623)	$P<10^{-4}$
2	ESA+CD44-	2/5	0/4	0/5	0/5			1/28963	(1/113477-1/7392)	
3*	ESA+CD44+			15/5	15/5	19/20	7/10	1/49	(1/76-1/32)	$P<10^{-4}$
3*	ESA+CD44-			5/5	2/5	0/5		1/568	(1/1202-1/268)	

→ **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 :**

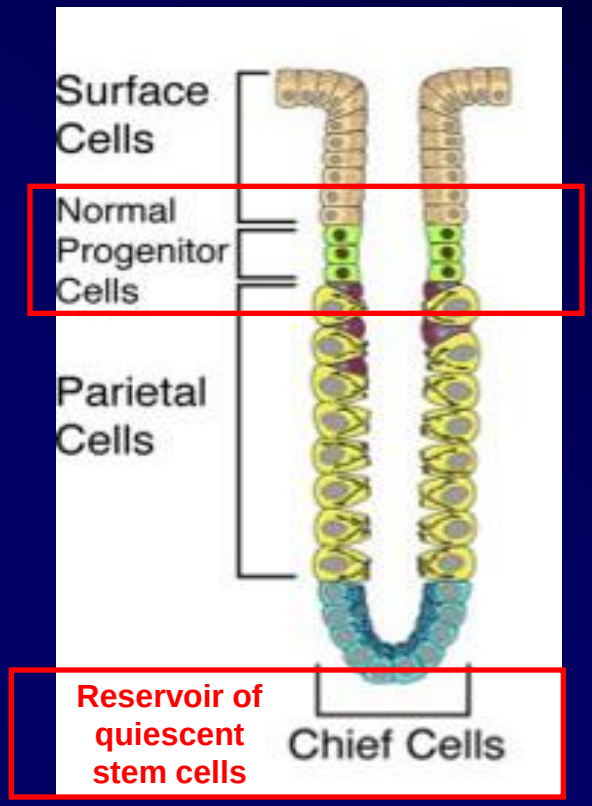
→ **1/ from local epithelial stem cells :**

- **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**
chief cells in corpus
signature (Lgr5 & CD44) (Stange *et al.* Cell 2013)



From Goldenring *et al*., Gastroenterology 2010

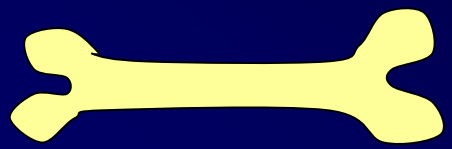
Cellular origin of gastric CSC ?

- **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)**



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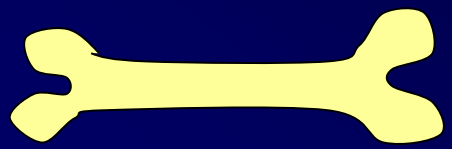
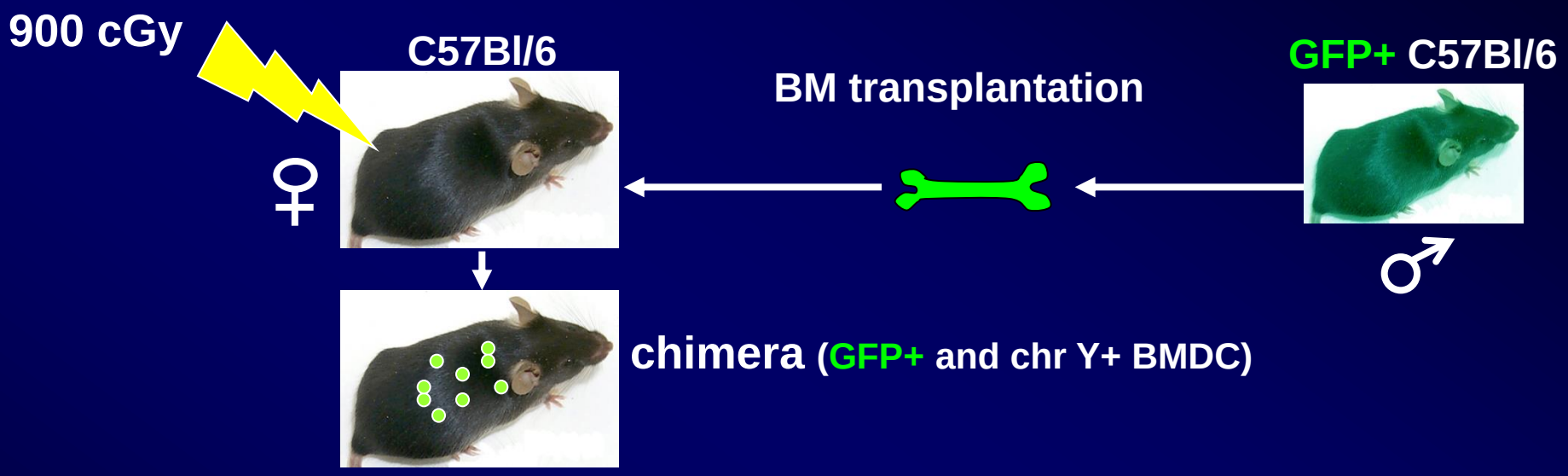
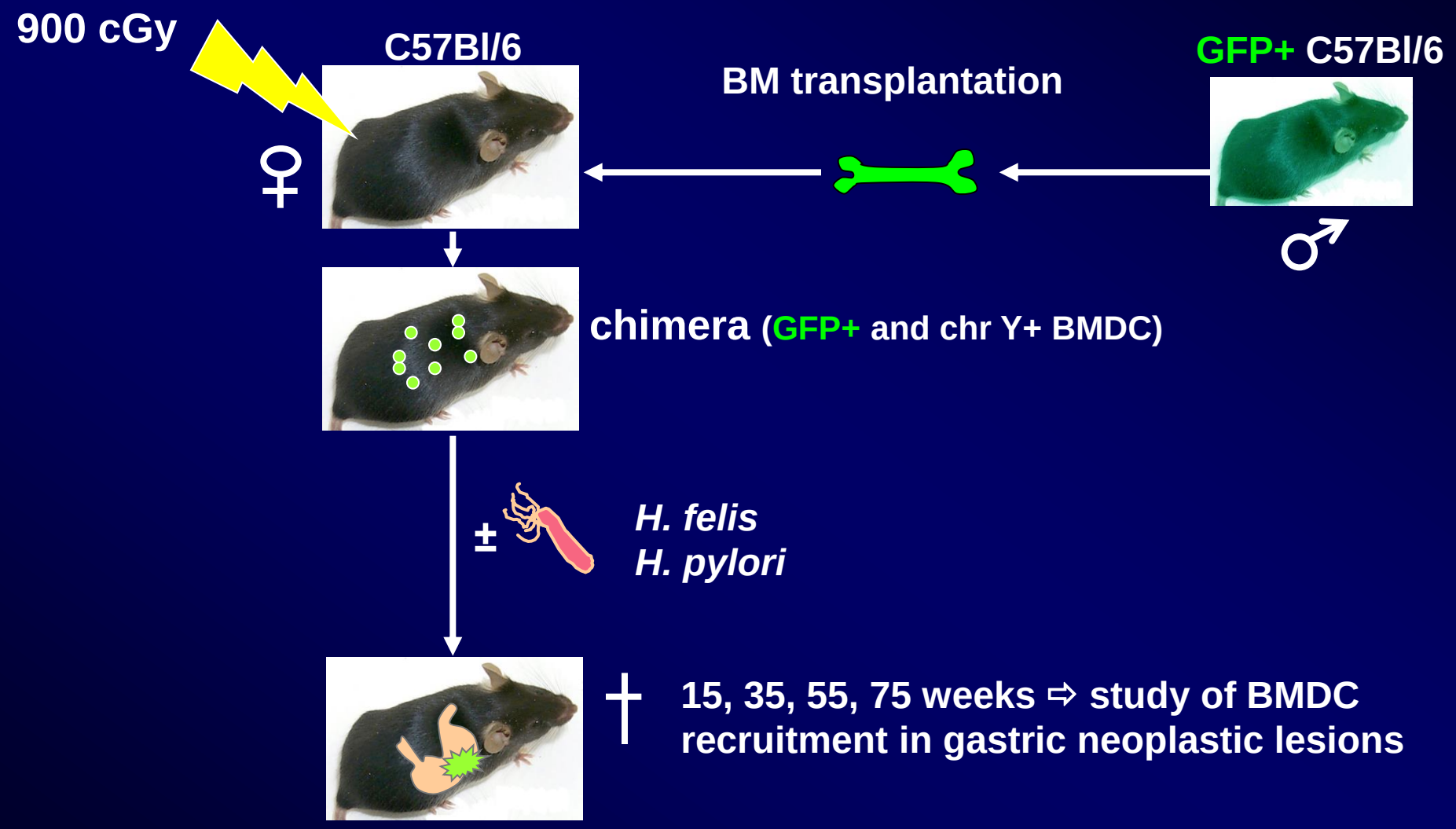


Is it also true in gastric carcinoma associated to the human pathogen *H. pylori* ?

Mouse model to study BMDC involvment in *H. pylori*-induced gastric carcinogenesis

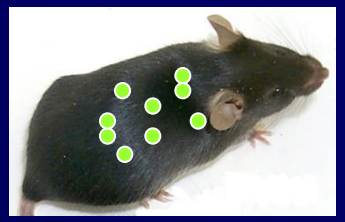


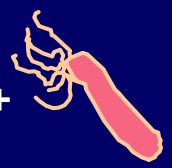
Mouse model to study BMDC involvment in *H. pylori*-induced gastric carcinogenesis



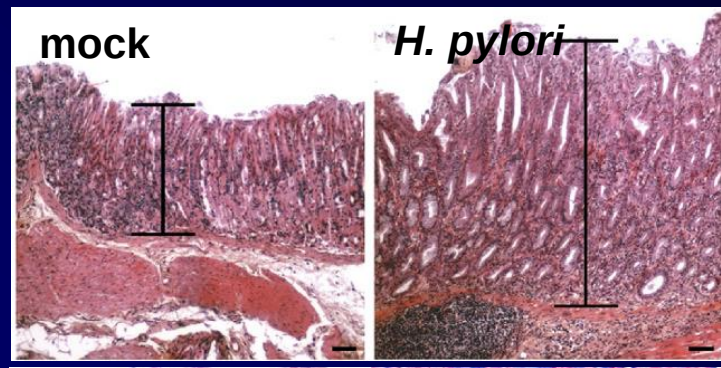
Varon *et al.*, Gastroenterology 2012

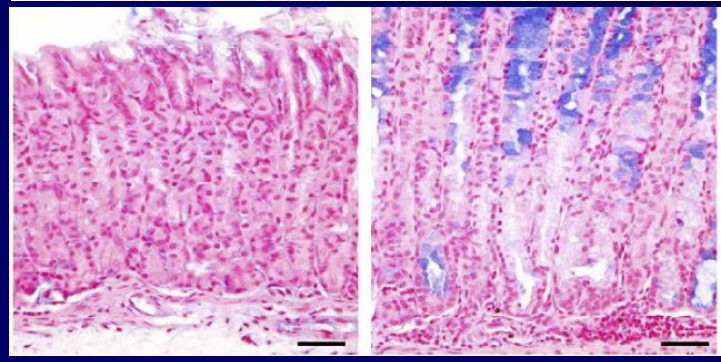
Mouse model to study BMDC involvment in *H. pylori*-induced gastric carcinogenesis



+ 

H. pylori
H. felis





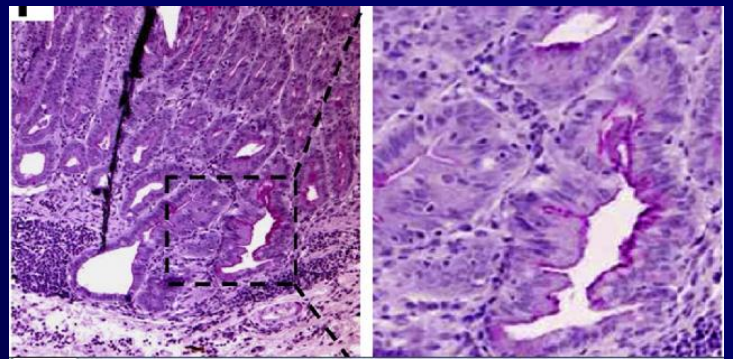
15 weeks — **Inflammation**

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

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

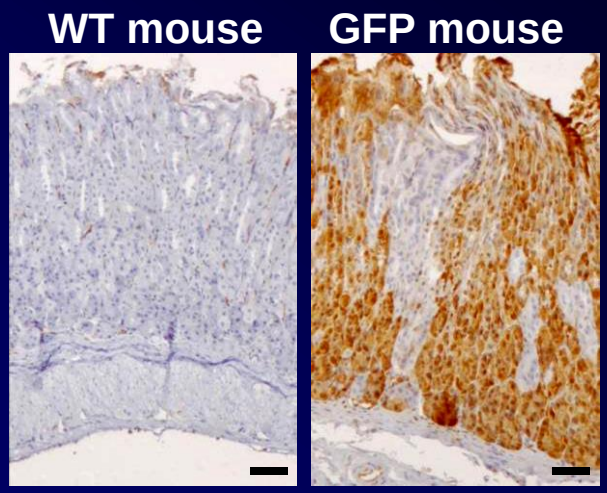
75 weeks

Dysplasia & Gastrointestinal Intra-epithelial neoplasia (GIN) :



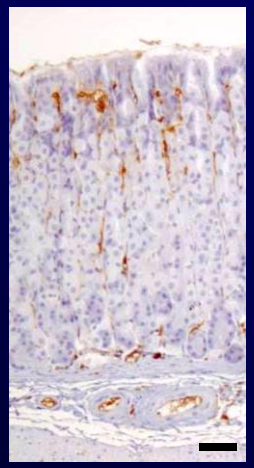
BMDC within neoplastic lesions induced by *H. pylori*

- Detection of BMDC by GFP immunohistochemistry on gastric mucosa:

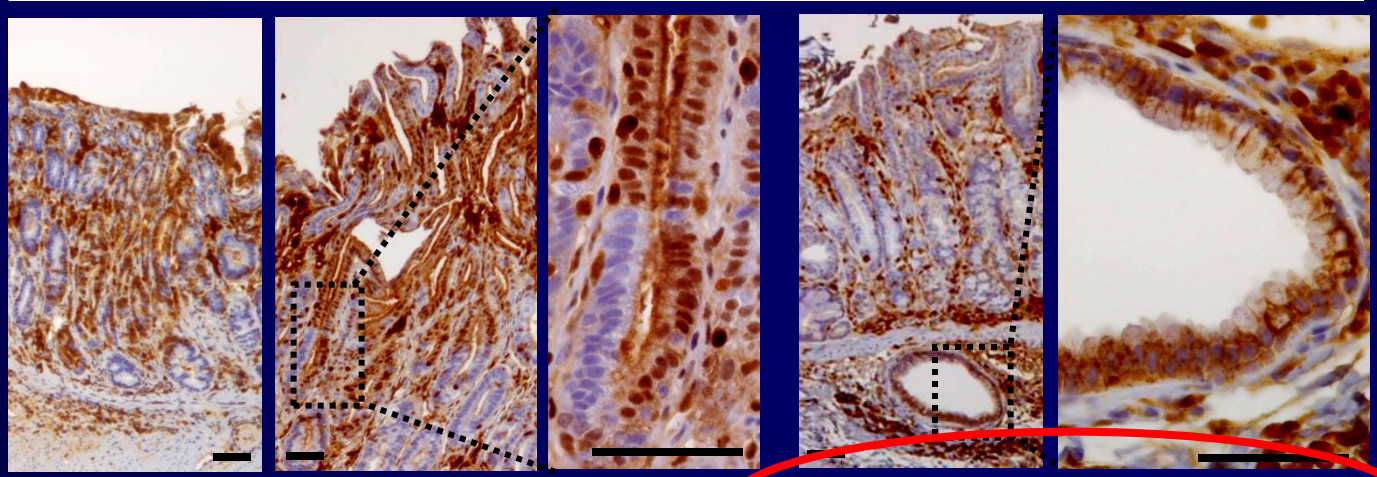


- Chimera after 1 year :

- H. pylori



+ H. pylori



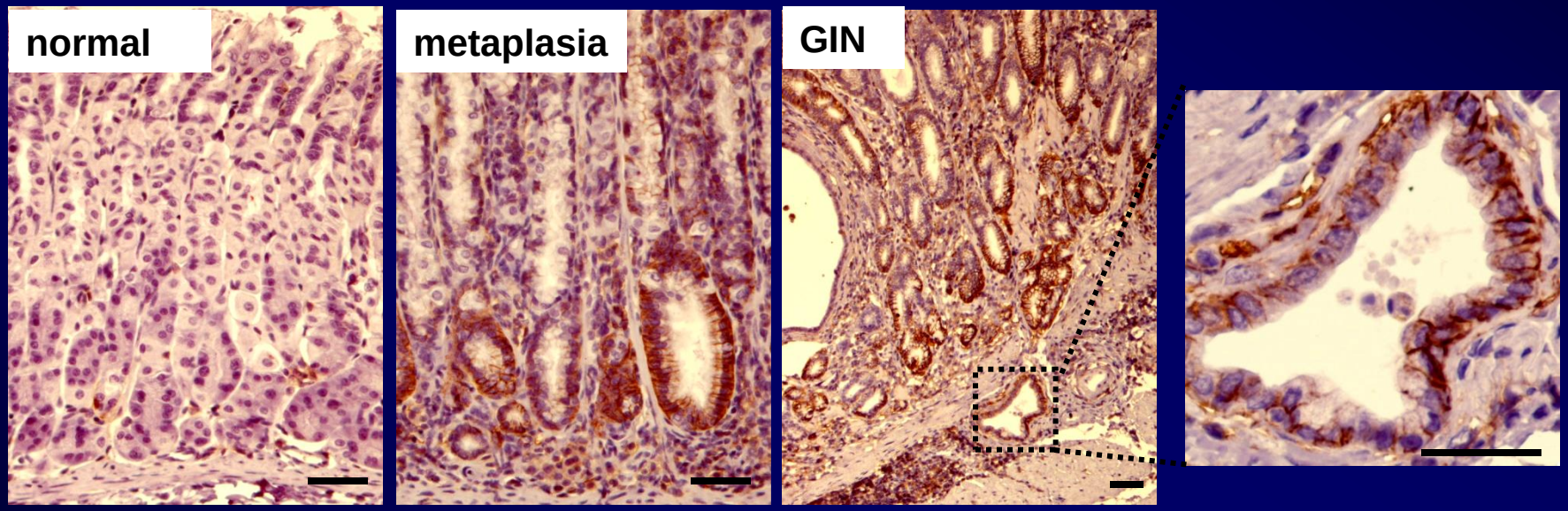
GFP+ pseudo-intestinal metaplasia

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* :



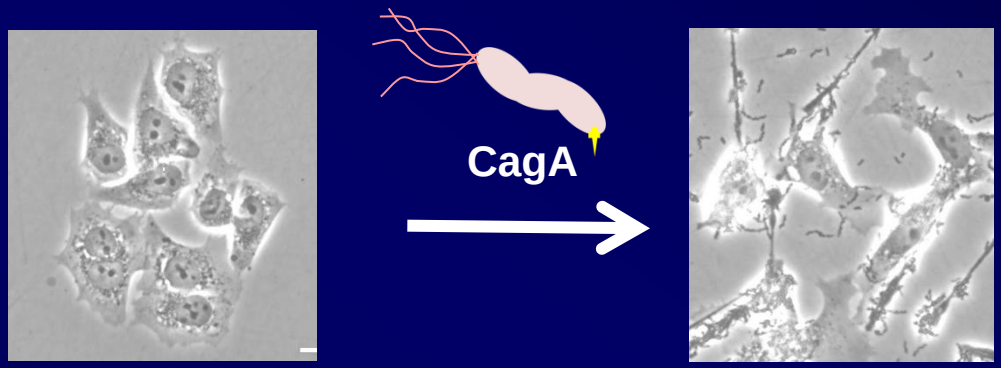
Varon *et al.*, Gastroenterology 2012

1 / Gastric CSC exist

2 / Origin of gastric CSC ?

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

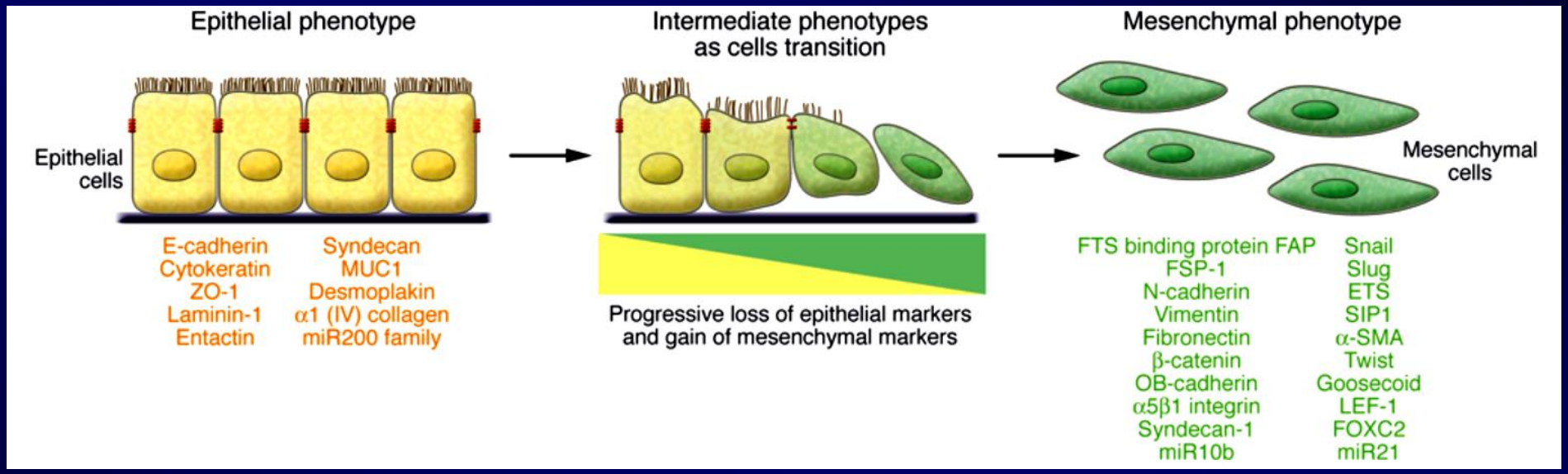
- **epithelial to mesenchymal transition**

Bagnoli et al., PNAS 2005

Yin et al., Gut 2010

Baud *et al.*, PLoS ONE 2013

The epithelial to mesenchymal transition (EMT)



Kalluri R. & Weinberg R., J Clin Invest 2009

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

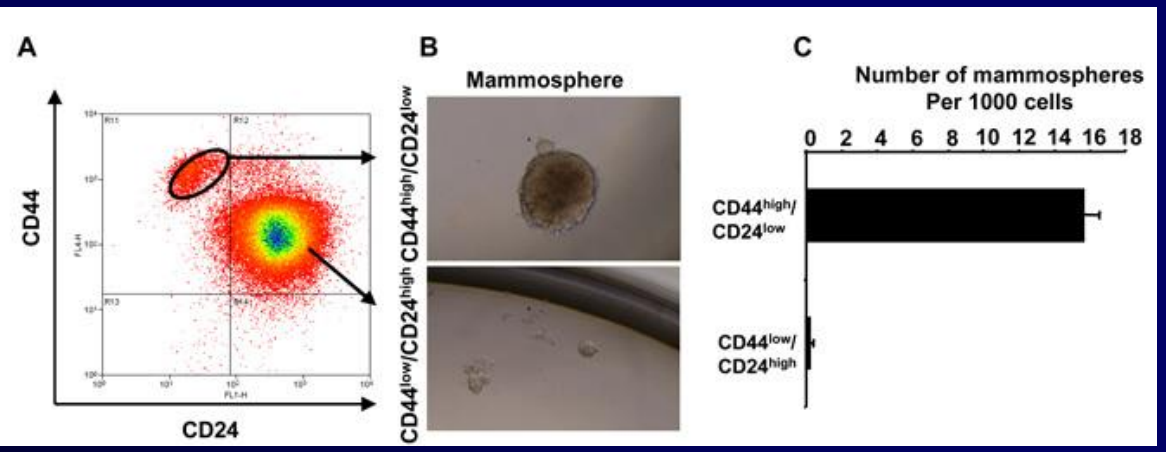
EMT induced in mammary epithelial cells generates cells with breast CSC properties

Immortalized mammary epithelial cells

↓ Snail or Twist overexpression

Induction of EMT

↓



Appearence of cells with the phenotype and properties of breast CSC

- $CD44^{high}/CD24^{low}$
- migration
- self-renewal
- tumorspheres *in vitro*
- tumors *in vivo*

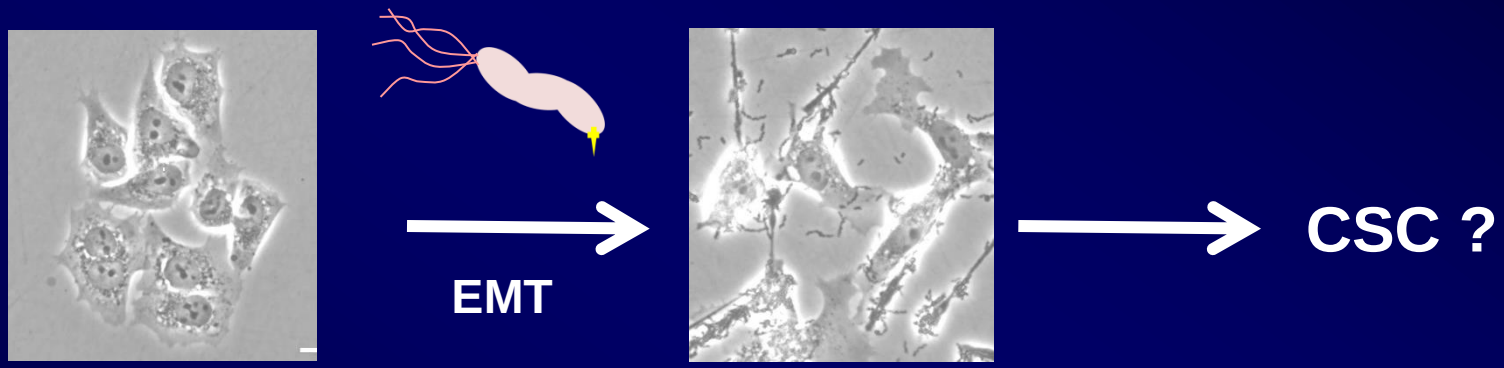
↓

EMT generates cells with CSC properties

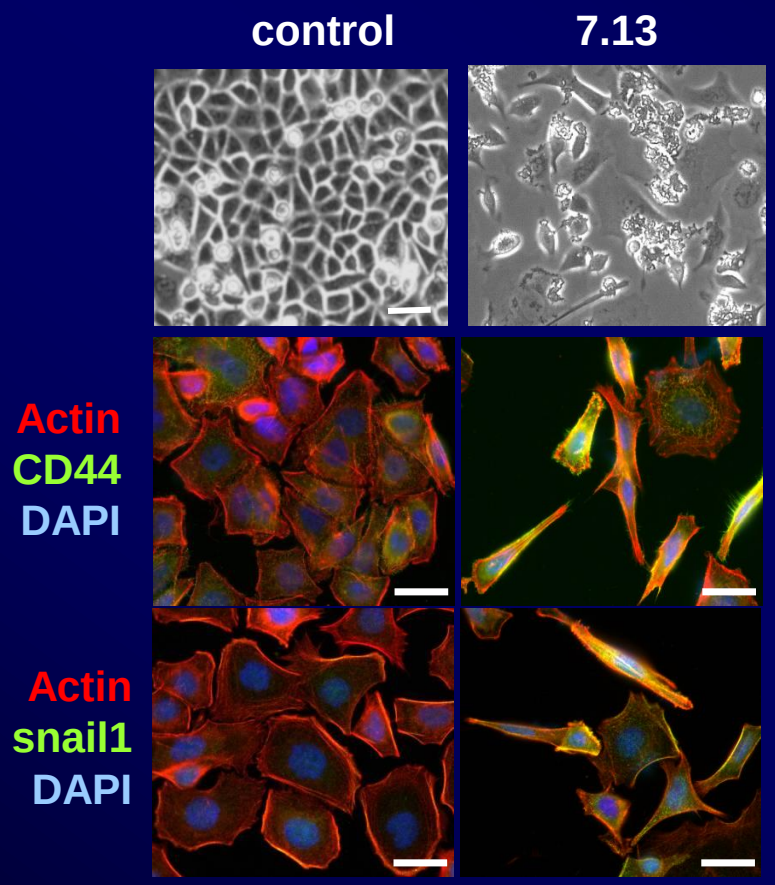
Morel et *al*., PloSONE 2008

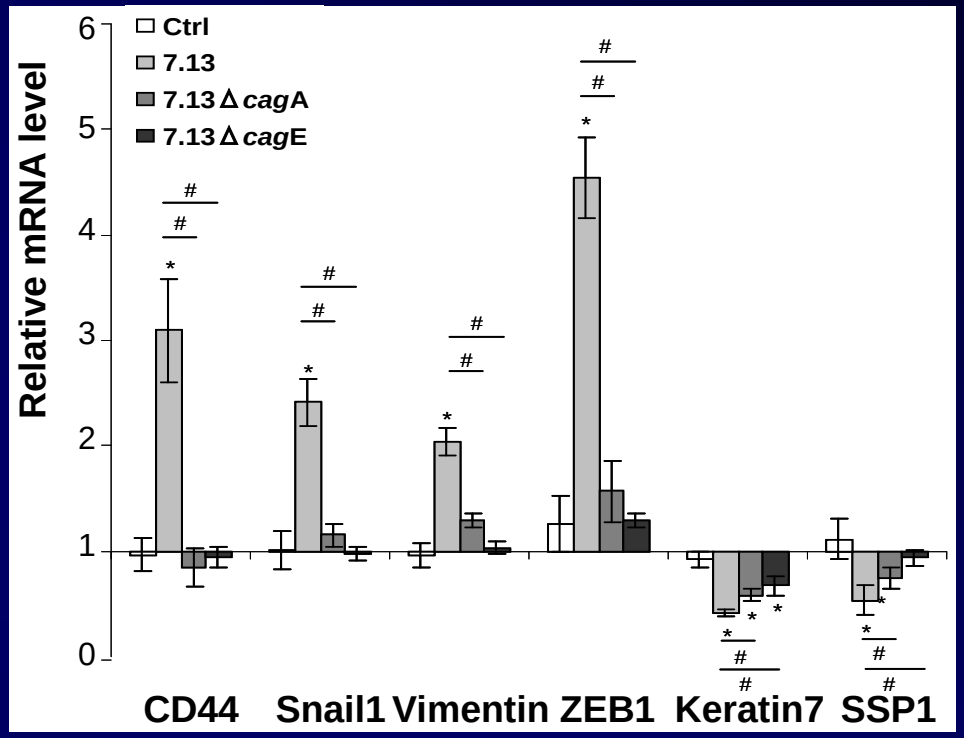
Mani et *al*., Cell 2008

Can *H. pylori*, via an EMT, generate cells with CSC properties ?



Cells harboring a mesenchymal phenotype in response to *H. pylori* infection are CD44^{high}



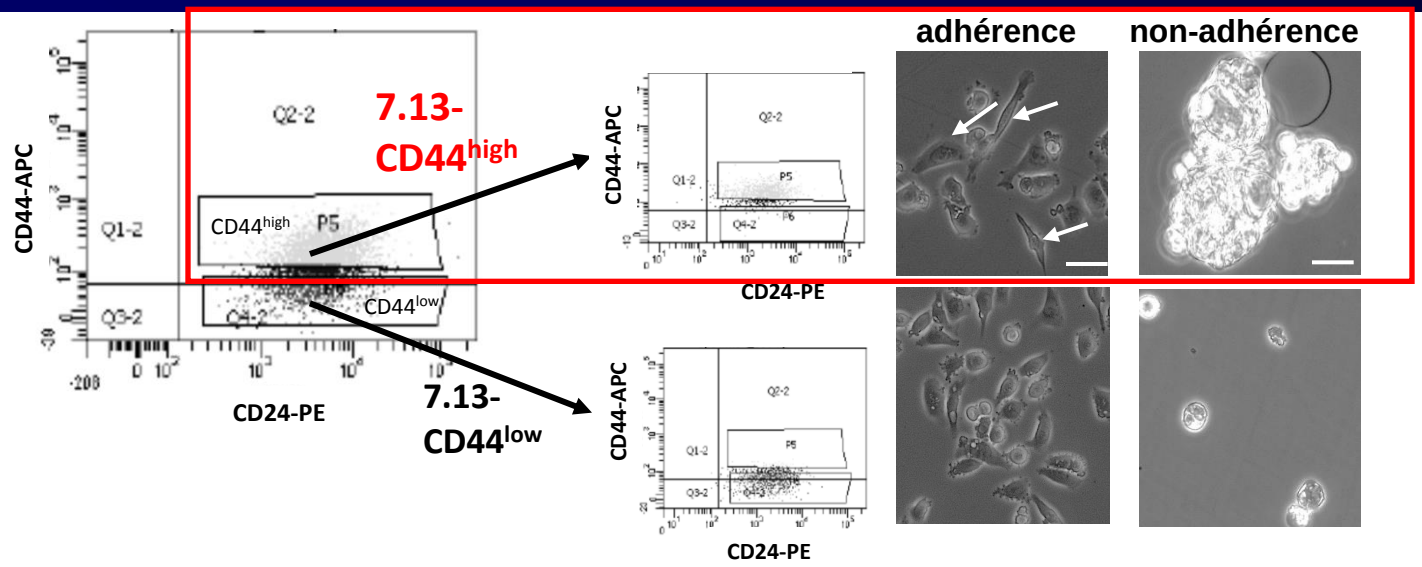


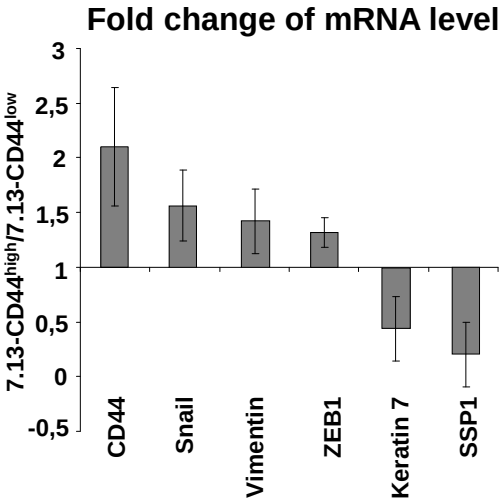
H. pylori **via CagA induces :**

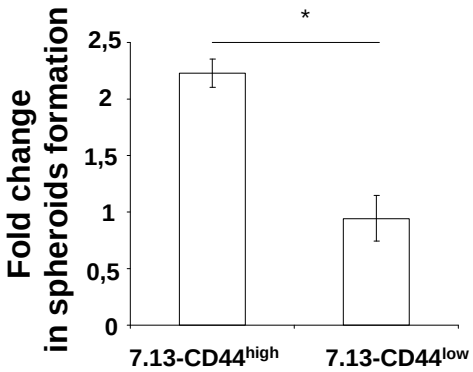
- **a mesenchymal phenotype**
- **molecular markers of the EMT**
- **cells harboring the mesenchymal phenotype overexpress CD44**

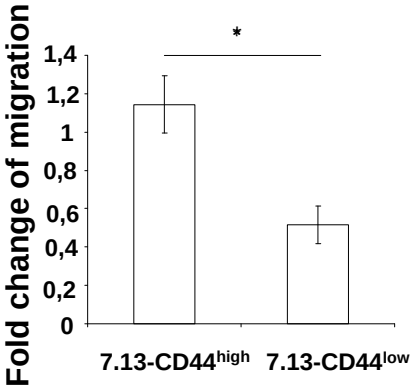
Bessède *et al.*, Oncogene 2013

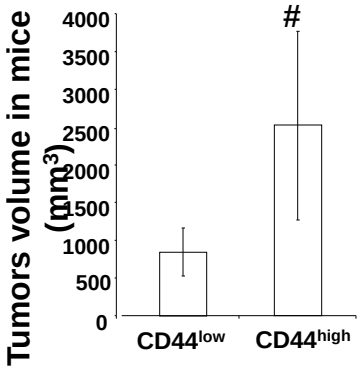
CD44^{high} cells induced by *H.pylori/CagA* harbor mesenchymal features and CSC properties





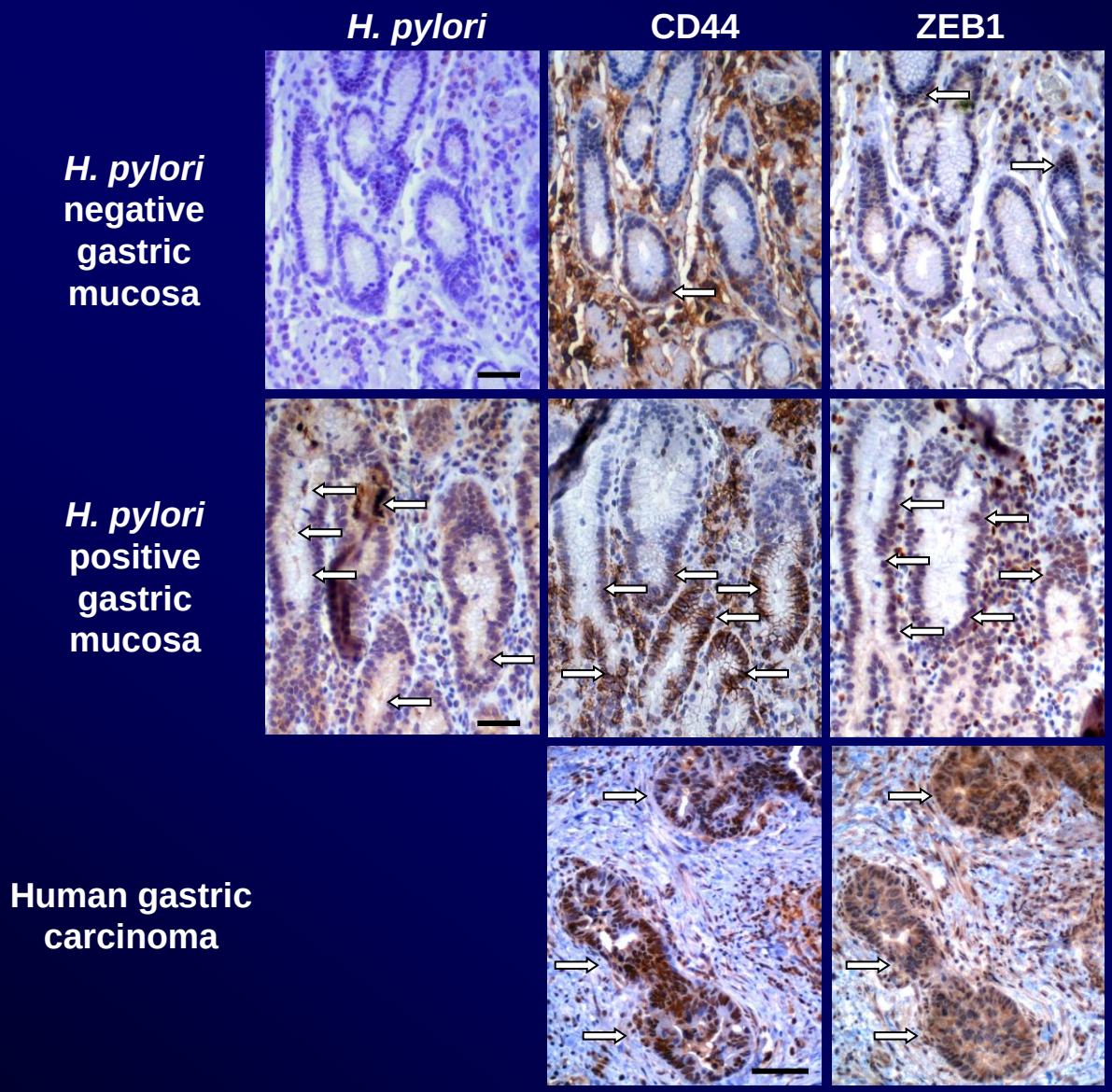






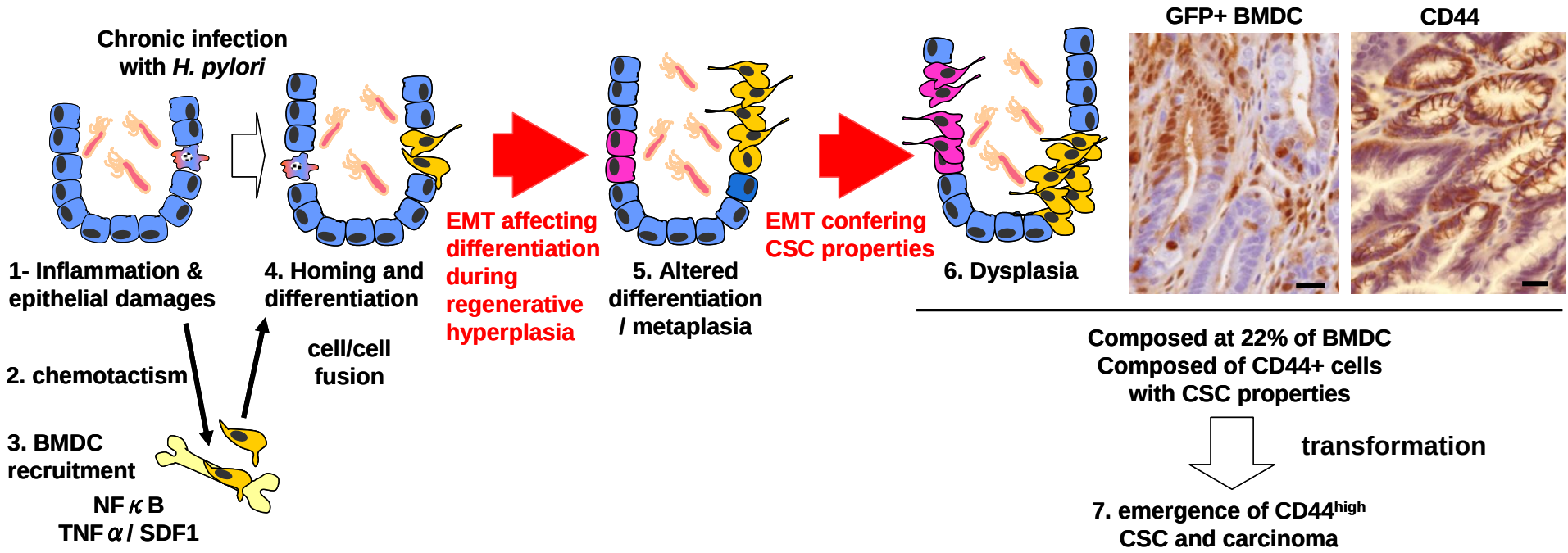
Bessède *et al.*, Oncogene 2013

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



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

INSERM U853 :

- F. Mégraud
- E. Bessède
- J. Ferrand
- L. Chambonnier
- PH. Nguyen (PhD)
- S. Molina (PhD)
- L. Acuna Amador

Collaborations :

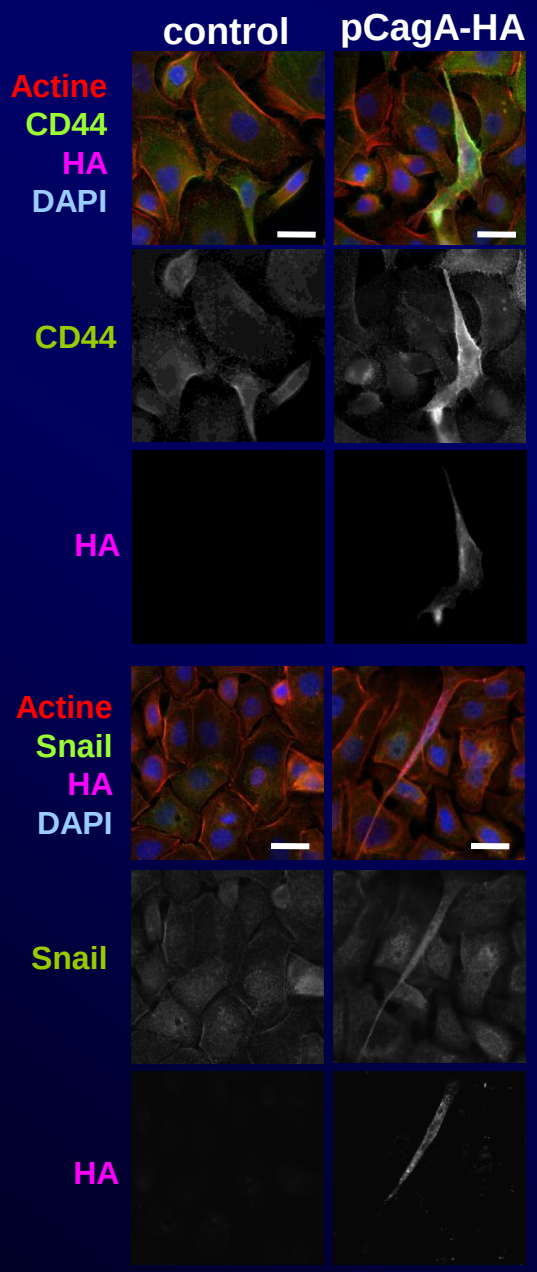
- **P. Dubus, A. Giese, M. Carlotti** (EA2406)
- **C. Staedel** (INSERM U869)
- **F. Mazurier** (INSERM U1035)
- **I. Soubeyran, S. Evrard** (Institut Bergonié)
- **G. Belleannée, D. Collet** (CHU Bordeaux),
- **L. Wittkop** (USMR, ISPED)
- **A. Schmidt-Alliana** (INSERM U576, Nice)
- **D. Noel** (INSERM U844, Montpellier)
- **M. Hatakeyama** (University of Tokyo, Japon)

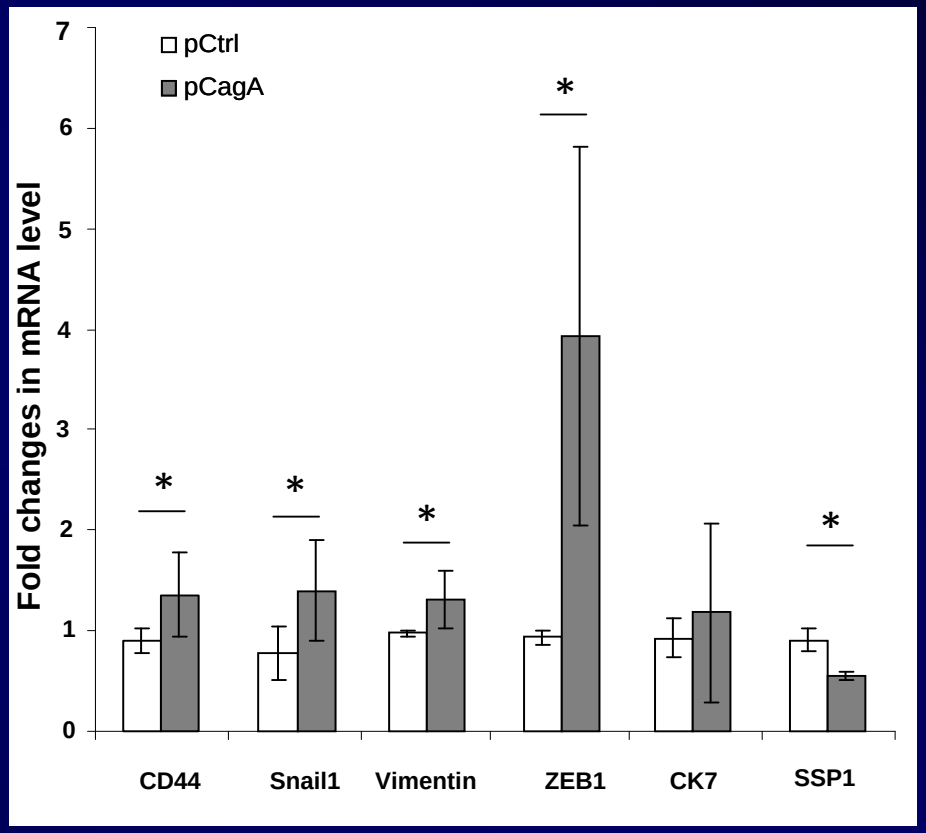
Platforms :

- **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

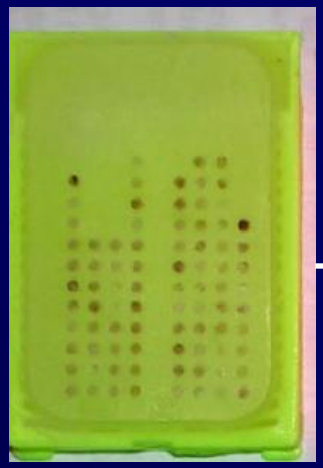
→ to confirm the expression of CD44 and markers of EMT and CSC in human tumors

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

250 cases : distal adenocarcinoma + distant non-cancerous mucosa

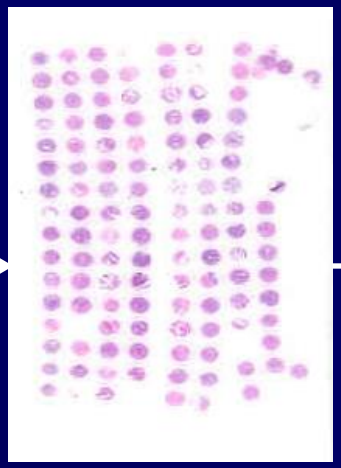
PET

Selection of areas of interest → **sampling (1 mm Φ)**



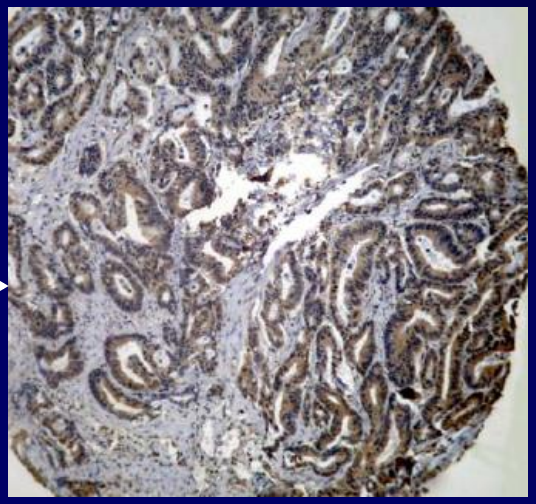
Paraffine embedding

→



IHC on section

→

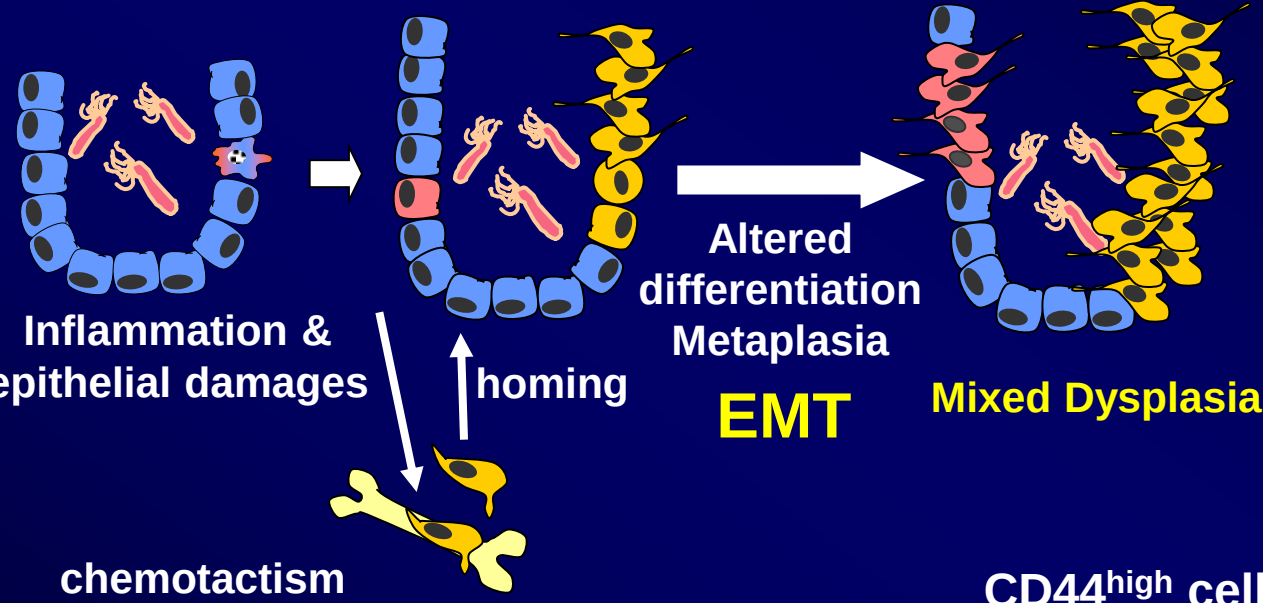


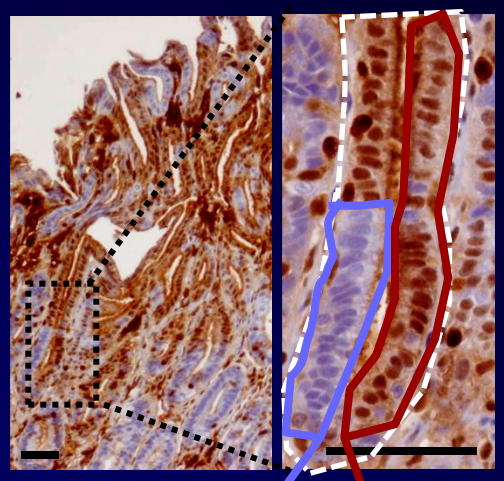
analyses

Varon et al. Methods in Mol. Biol. 2009

→ **Markers of pre-neoplastic lesions? Predictive of severity, prognosis?**

Cellular and molecular determinants of *H. pylori*-induced gastric carcinogenesis





CD44^{high} cells with CSC properties

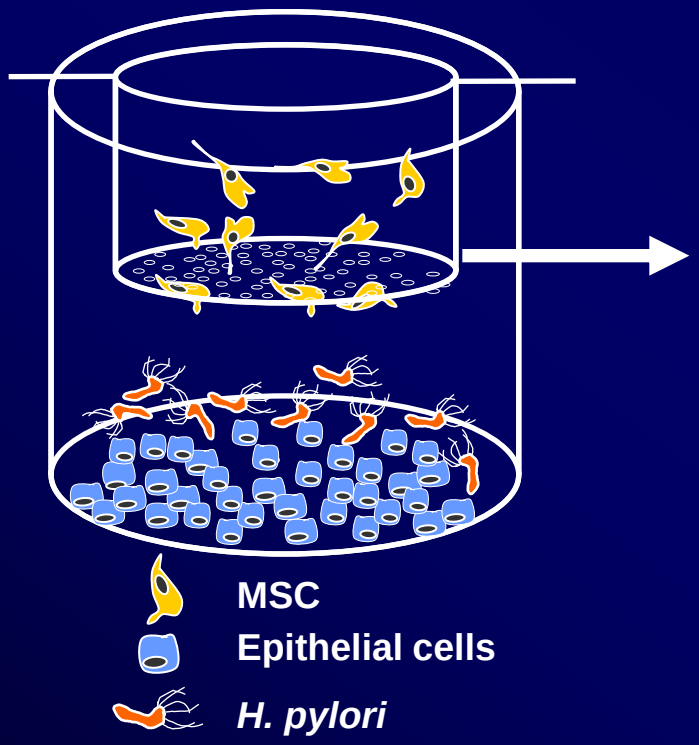
Transformation

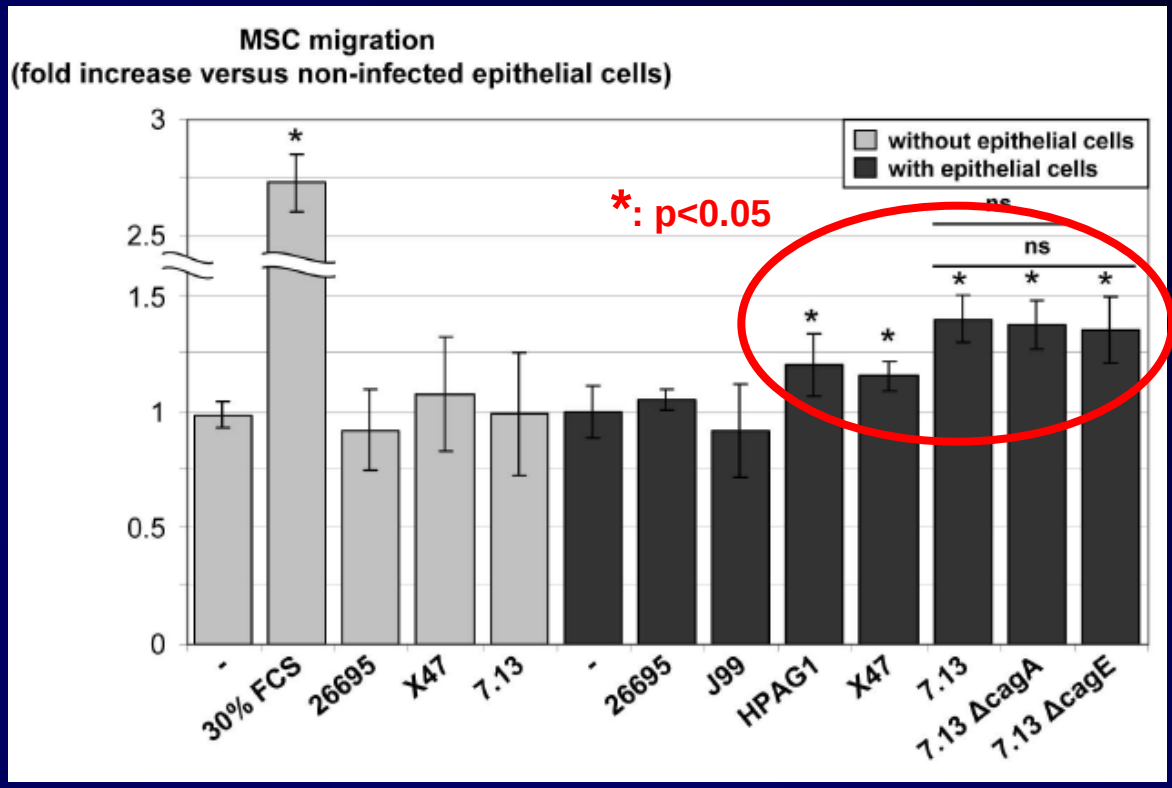
adenocarcinoma
CD44^{high} CSC

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

H. pylori and BM-MSC recruitment

- **Migration assay :**

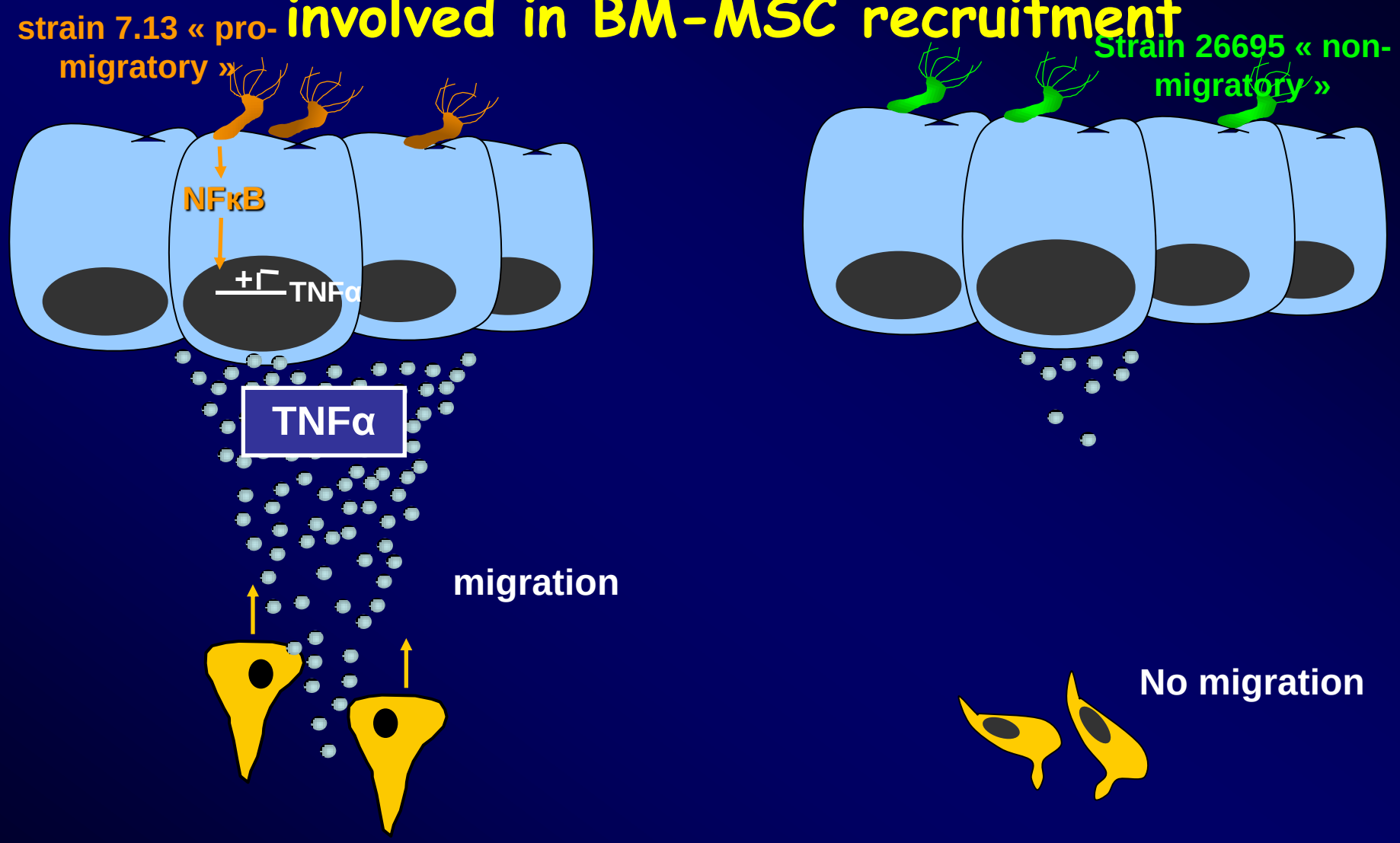




- ⇨ **Some strains of *H. pylori* stimulate MSC migration**
- ⇨ **No association with the *cag*PAI or VacA status of strains**
- ⇨ **Associated to the capacity of strains to induce epithelial cells apoptosis**

Ferrand *et al.*, PLoSOne 2011;6(12):e29007

Identification of chemokines involved in BM-MSC recruitment



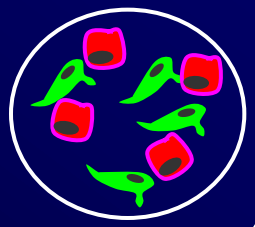
→ *H. pylori*-induced MSC migration depends on NFκB activation and secretion of TNFα

Ferrand *et al.*, PLoSOne 2011;6(12):e29007

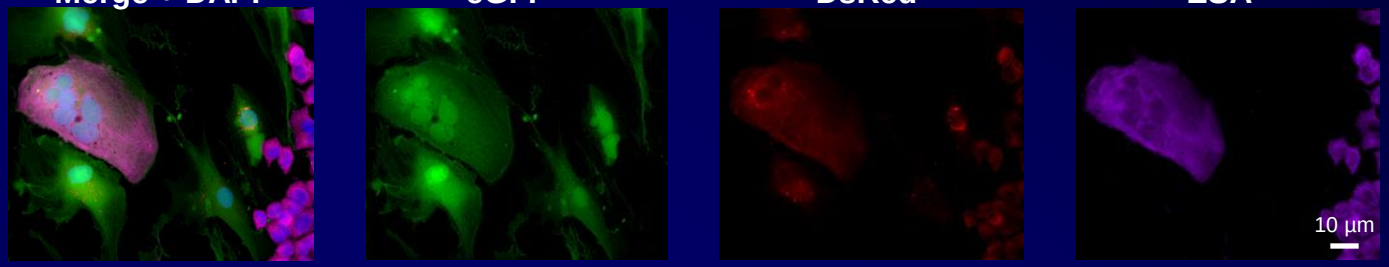
BM-MSC acquire an epithelial phenotype through cell fusion *in vitro*

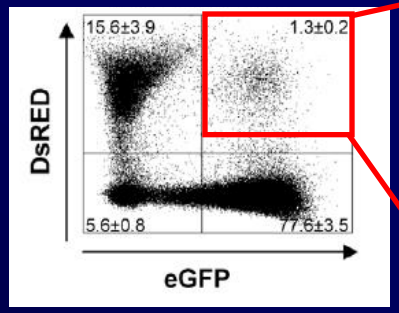
BM-MSC eGFP

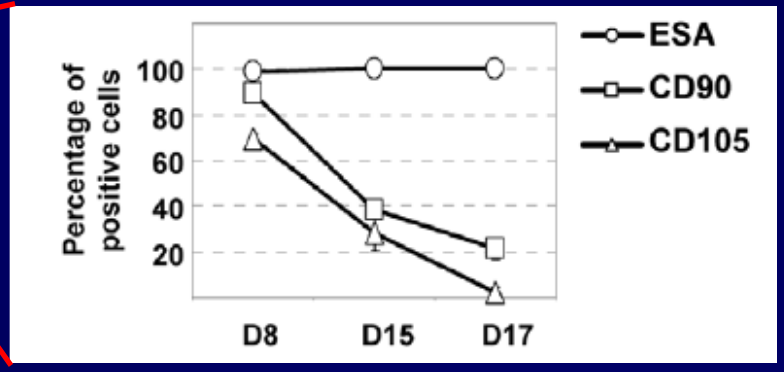
gastric epithelial cells DsRED (AGS)



8 days







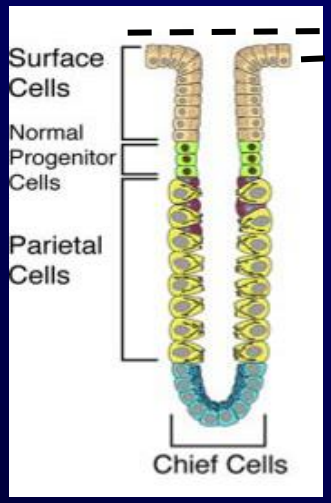
→ 1.3% of fused cells after 8 days of coculture

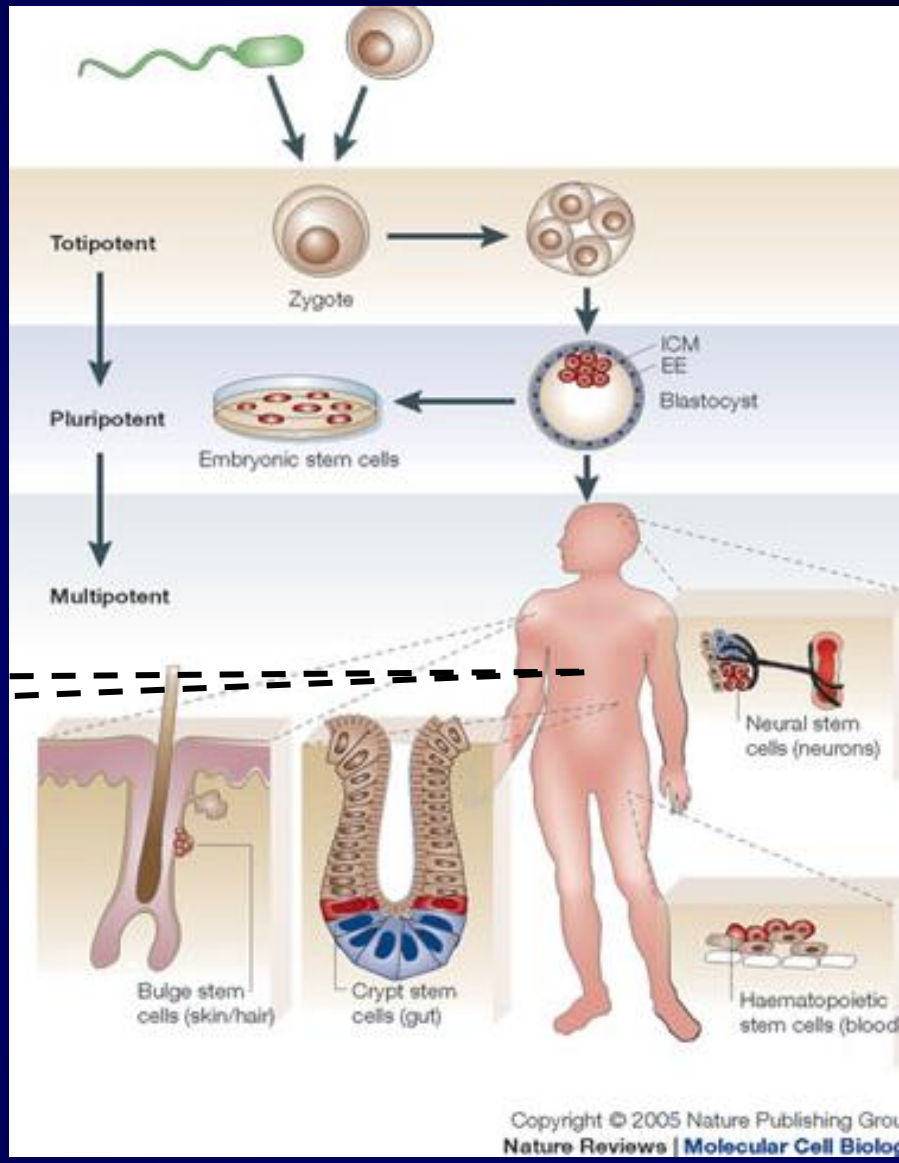
→ loss of mesenchymal markers, gain of epithelial markers

Ferrand *et al.*, PLoSOne 2011;6(5):e19569

Stem cells in adult tissues

- Self-renewal
- Asymetrical 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)