Corso di Biologia Cellulare del Cancro AA 2019-2020

Dormienza e colonizzazione metastatica

The seed and soil hypothesis

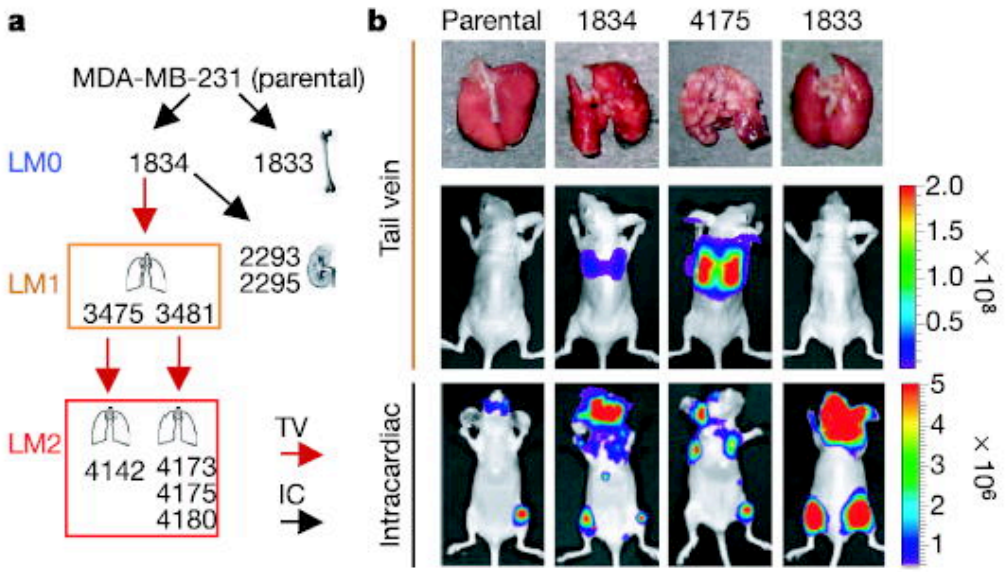
"What is it that decides what organs shall suffer a case of disseminated cancer?"

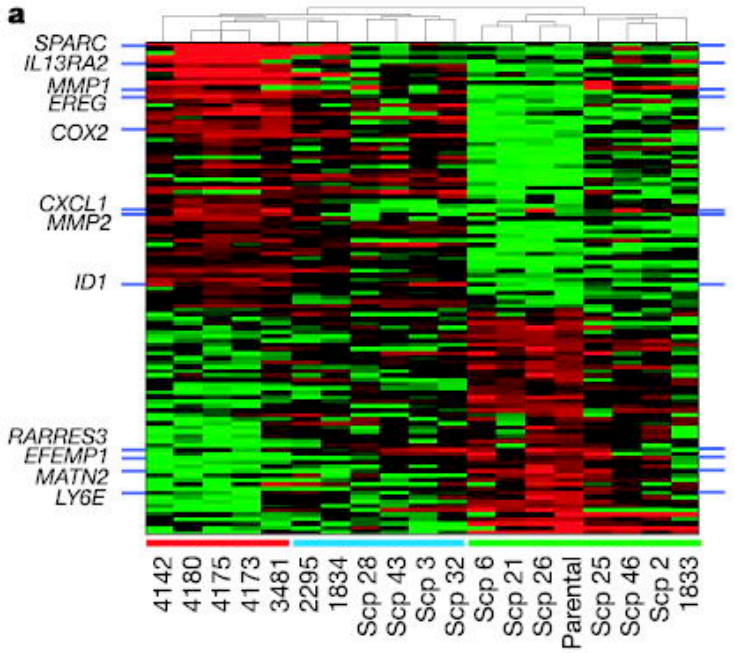
"When a plant goes to seed, its **seeds** are carried in all directions….,but they can only live and grow if they fall on congenial **soil**."
-Stephen Paget 1889-

Seed: i geni della metastasi

Metastasis develops through genetic and epigenetic changes and the subsequent selection for favourable traits under the pressure of successive bottlenecks.

Seed: i geni della metastasi





Flow chart of the selection of organ-specific metastatic subpopulations in vivo, indicating the organs from which these subpopulations were isolated. Each **subsequent lung-metastatic generation** is designated LM0, LM1 and LM2. The LM2 cells were further analysed for metastasis by either tail-vein (TV) or intracardiac (IC) xenografting.

Gene-expression signature associated with lung metastasis
a, Comparison of gene expression profiles of LM2 populations with parental cells identifies 113 probe sets that are correlated with lung metastatic activity.

Seed: i geni della metastasi

Vol 459|18 June 2009|doi:10.1038/nature08021 nature

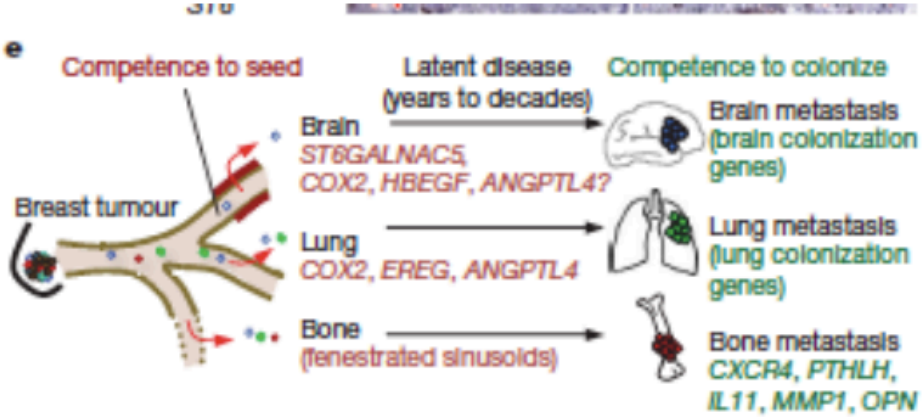
LETTERS

Genes that mediate breast cancer metastasis to the brain

Paula D. Bos[1], Xiang H.-F. Zhang[1], Cristina Nadal[1]†, Weiping Shu[1], Roger R. Gomis[1]†, Don X. Nguyen[1], Andy J. Minn[2], Marc J. van de Vijver[3], William L. Gerald[4], John A. Foekens[5] & Joan Massagué[1,6]

***in-vivo*-selected brain metastatic derivatives**

Gene expression analysis of these cells and of clinical samples+ functional studies:
cyclooxygenase COX2 (al known as PTGS2), the epidermal growth factor receptor (EGFR) ligand HBEGF, and the a2,6-sialyltransferase ST6GALNAC5 **as mediators of cancer cell passage through the blood–brain barrier**



Origine della competenza metastatica

Genomic comparisons show close clonal relationships between primary tumours and their metastases. Specific ancestors of metastatic clones can often be identified in the primary tumour, supporting the **hypothesis that late a clonal expansion in the primary tumour gives rise to metastasis competent clones**. These studies also provide evidence for **metastases seeding new metastases**.

Disseminated cancer cells remain dependent on the oncogenic mutations that underlie the primary tumour, providing a basis for **treating metastasis** with drugs that target these oncogenic drivers.
In line with this observation, **gains in oncogenic mutant alleles occur in metastases**, including gains in mutant **KRAS** in pancreatic cancer metastasis, and **TP53** and androgen receptor mutations in prostate cancer metastasis.

To date, however, **no recurrent metastasis-specific mutations have been identified**, suggesting that **epigenetic** alterations and other sources of **modified gene expression** are the predominant **source of selectable pro-metastatic traits** during clonal evolution in metastasis.

The **cell-autonomous traits** that favour cancer cell dissemination, resistance in circulation, extravasation, and initial survival in distant organs matter immediately after cancer cells depart from the primary tumour, and **are pre-selected in the primary tumour**. For example, certain mediators of **neoangiogenesis** in breast tumours, including **COX2, MMP1, and VEGF are repurposed by cancer cells for extravasation** in the lungs and brain. **Stromal TGF-β** in the triple-negative subset of breast carcinomas induces expression of **ANGPTL4** in cancer cells, thus priming these cells for extravasation in the lungs. These **early metastatic traits** may be **selected under the stresses** of tissue invasion, immune surveillance, or hypoxia.

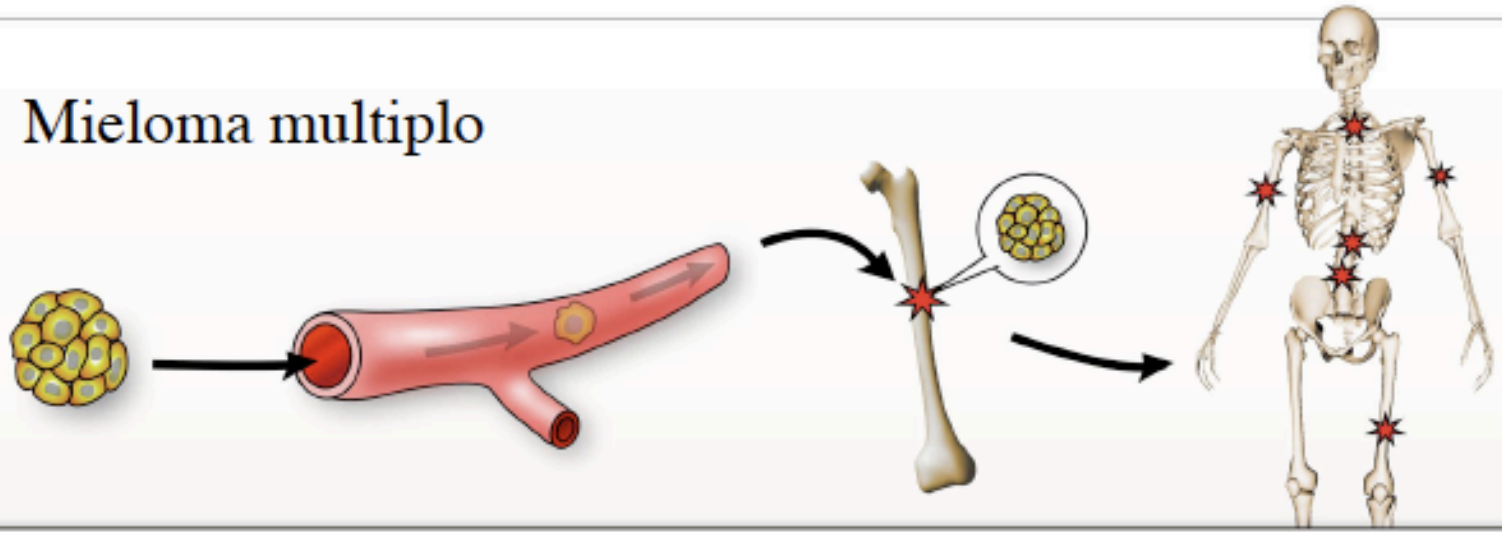
The evidence favours a **model in which a significant proportion of cancer cells in a primary tumour acquire pro-metastatic traits that confer a finite probability of success in the early steps of metastasis**. Clones with the most effective combination of pro-metastatic traits stand the highest probability of giving rise to metastatic lesions, and also to **re-seeding** the primary tumour.

Beyond these early steps, **cancer cells continue to evolve after dissemination** to distant organs, **acquiring traits for overt colonization** as suggested by the case of bone metastasis. Cells that disseminate early from a tumour could evolve in parallel with, but independently from the primary tumour.

The origin of metastatic traits remains a fertile area for future research.

Massagué and Obenauf.

Mieloma multiplo



Primary tumor; Local invasion; Plasmacytoma	Circulation	Micrometastasis; MGUS	Colonization; Multiple lytic lesion-symptomatic disease

Genes regulating tumor initiating function:
Cyclin D1/D3, FGFR3, MMSET, c-maf, del 13q

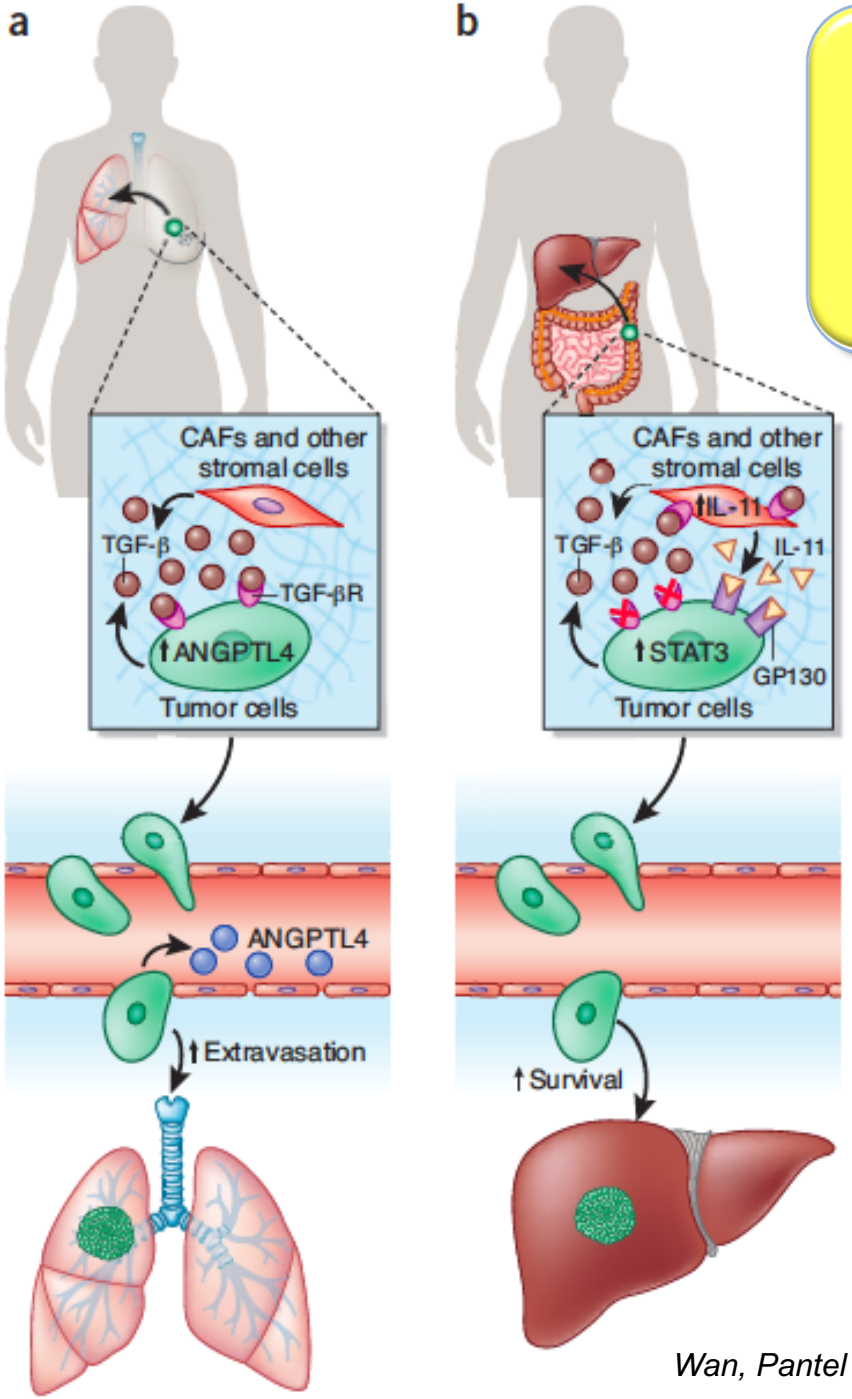
Metastasis initiating function: Angiogenesis, invasion and circulation; EMT- transcription factors, hypoxia-regulated genes, CXCR4

Metastasis progression:
Activation of NFκB, N-ras, K-ras, FGFR3

Metastasis virulence:
PTHRP, IL-6, TNFα, VEGF, RANKL

Seed: i geni della metastasi

L'interazione con il microambiente del tumore primario influenza la successiva metastasi

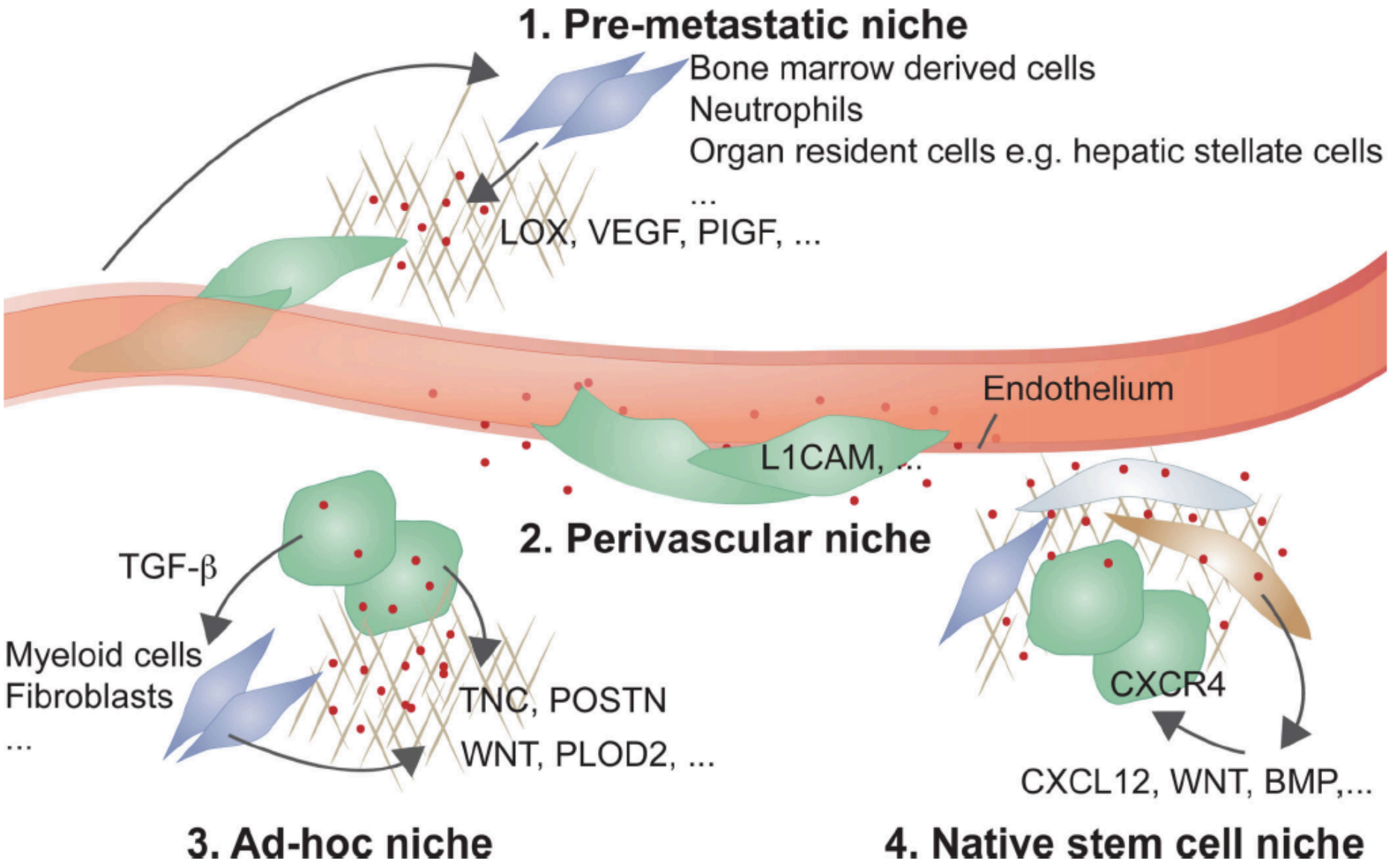


"Seed and soil" theory (ipotesi di Stephen Paget (1855 – 1926)):
il "seed", cioè la cellula tumorale, deve essere compatibile con il "soil", cioè la nicchia pre-metastatica dell'organo che sarà infiltrato.

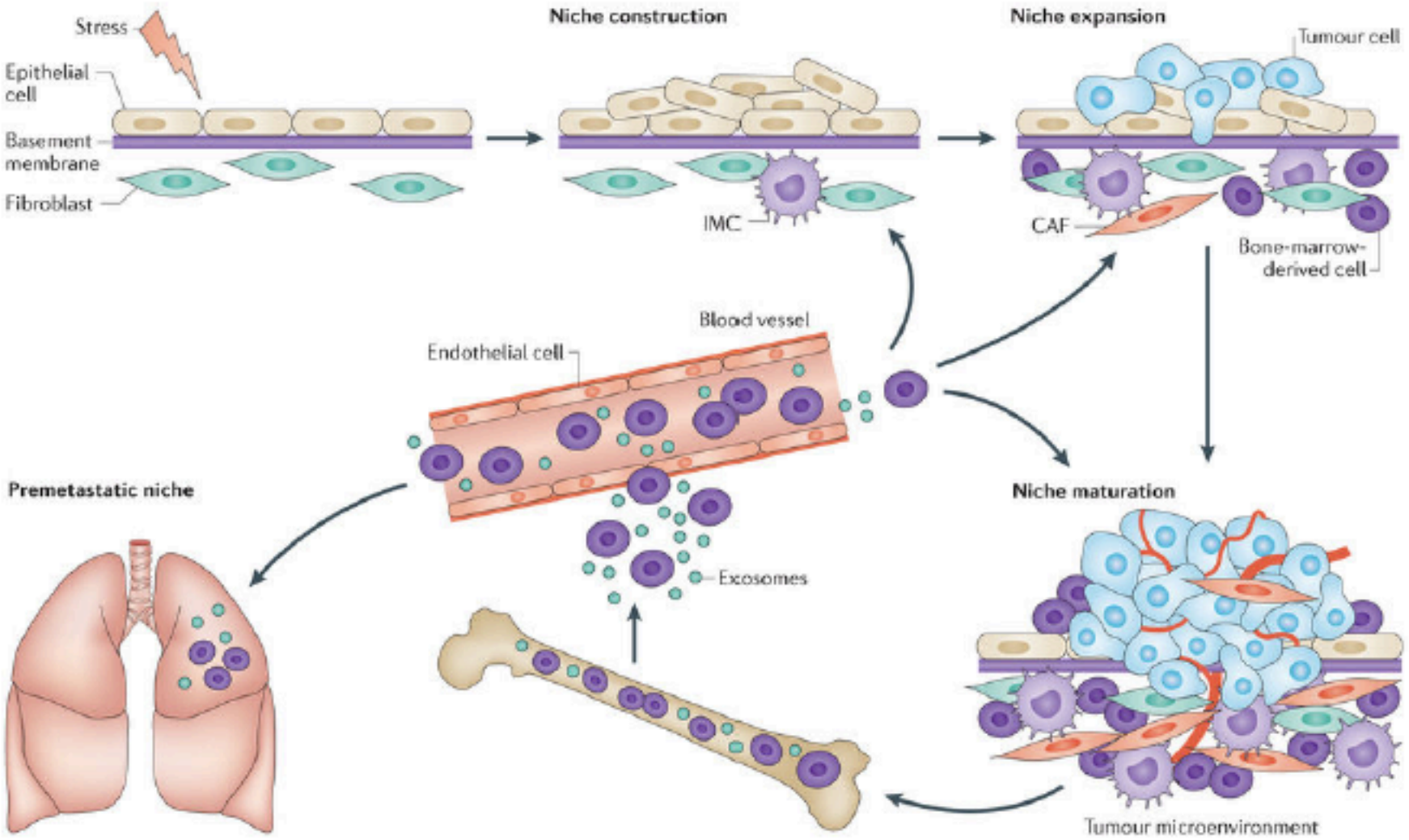
Importanti cambiamenti vengono indotti dall'interazione tra la cellula tumorale ed il nuovo microambiente, a formare la nuova nicchia.

Wan, Pantel & Kang, Nat Med 2013

Soil: la NICCHIA metastatica



La costruzione della NICCHIA metastatica



Nature Reviews | Cancer

Microambiente e dormienza metastatica

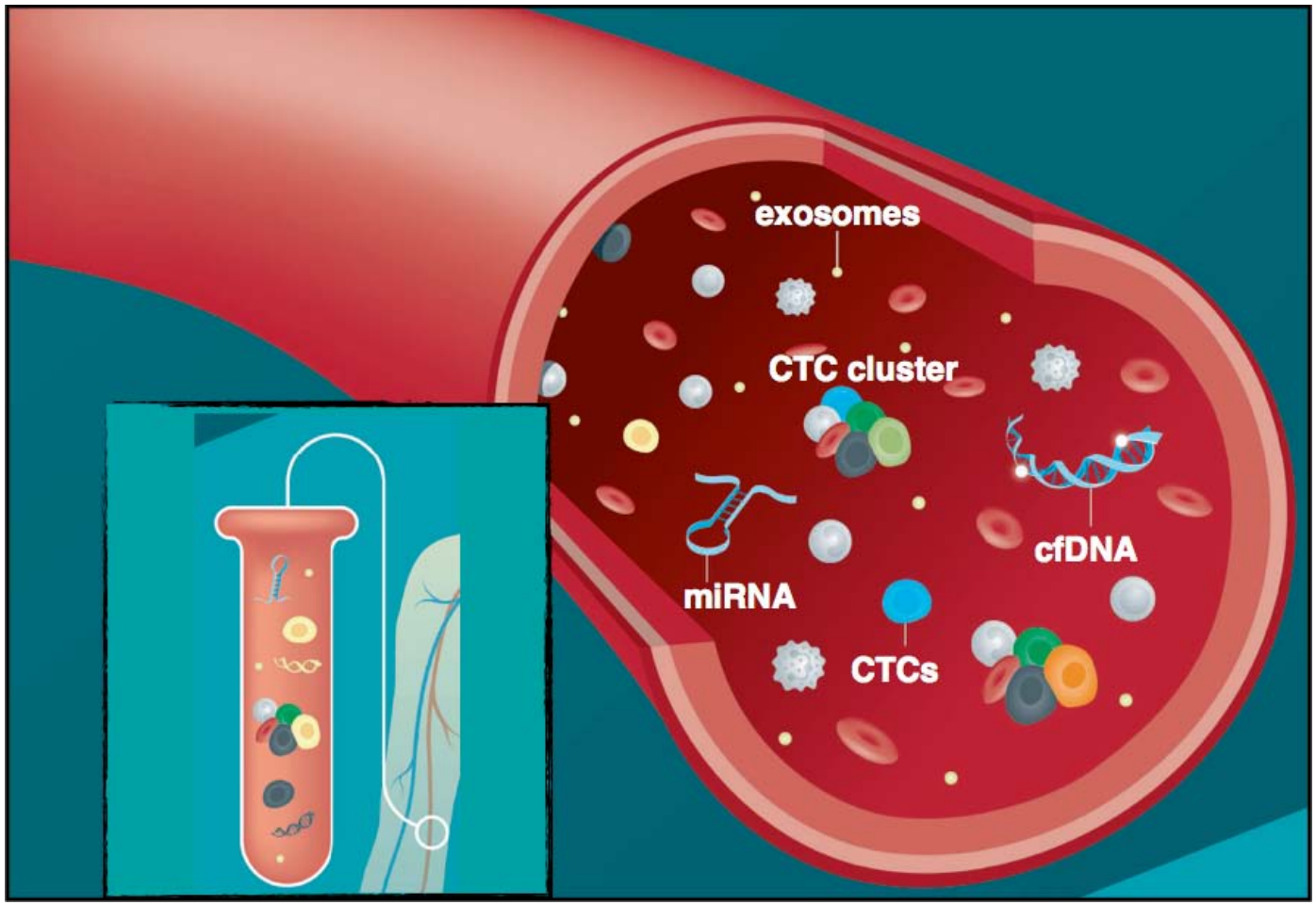
• Cells that extravasate seem almost invariably destined to **either be eliminated from the tissue** parenchyma or **enter into a state of dormancy in which they persist in an indolent state as single disseminated tumor cells (DTCs) or as small micrometastatic clusters—for weeks, months, years**

• The entrance into this growth-arrested state may often be **attributable to a microenvironment to** which these cells are poorly adapted when they first arrive after extravasation.

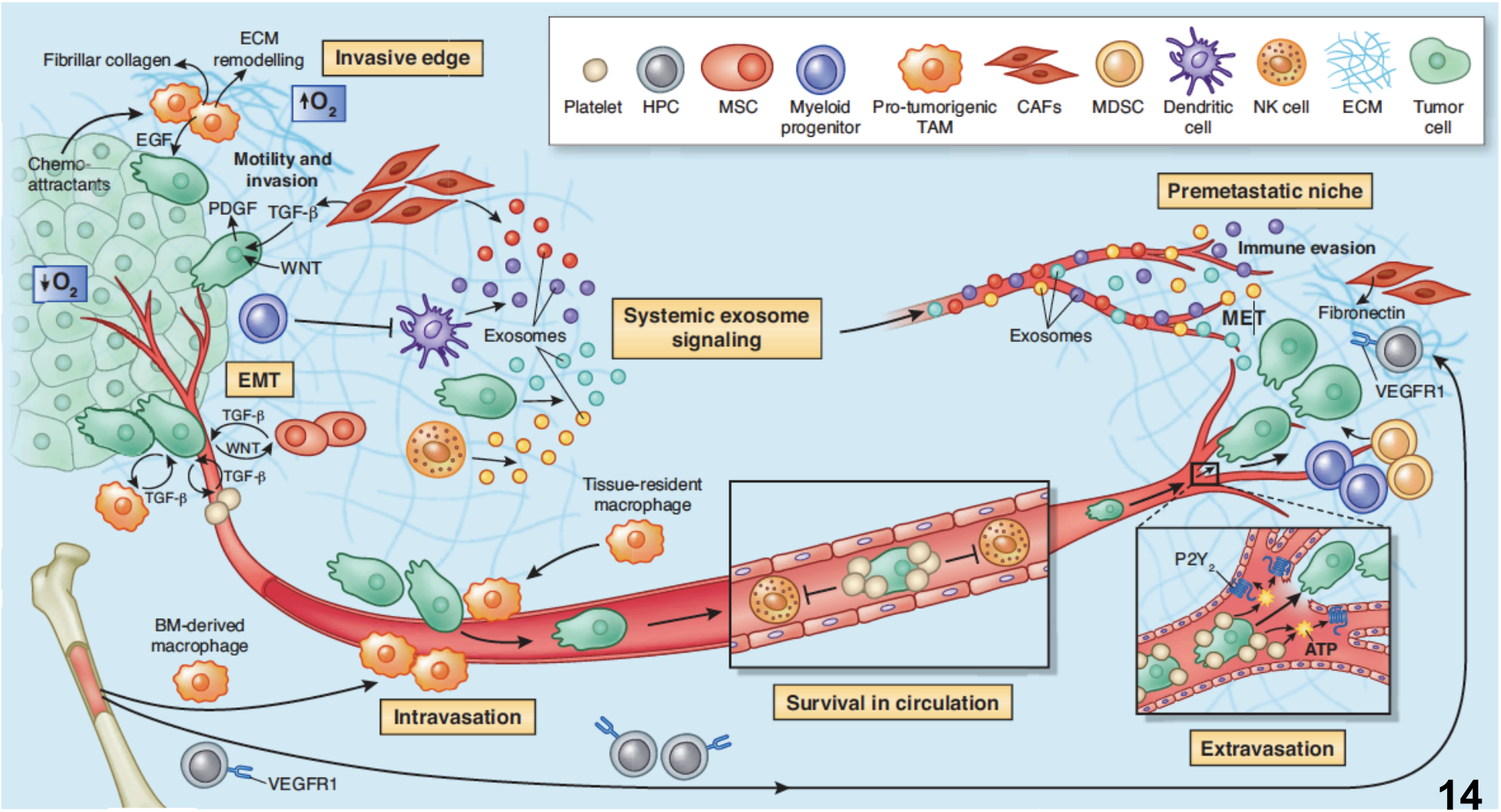
• a **dormant state can also be actively imposed by certain anti-proliferative signals encountered** by recently arrived cells in the parenchyma of foreign tissues.

• Dormant DTCs may reside in **specialized niches that support their survival, block proliferation, and** possibly **provide resistance to therapeutic agents. Dormant DTCs can co-opt a niche otherwise** reserved for tissue-resident stem cell populations. In many organs DTC may reside close to the microenvironment surrounding the vasculature (**perivascular niche).**

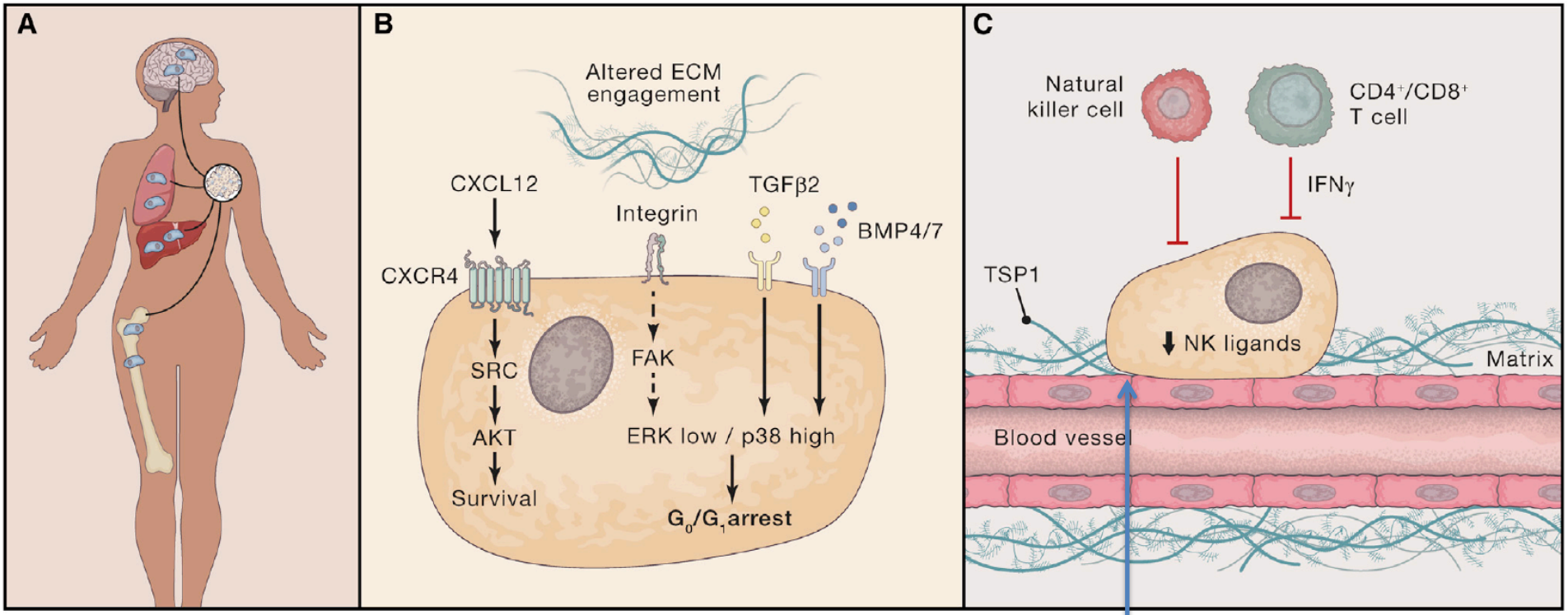
EDUCAZIONE della NICCHIA pre-metastatica da fattori secreti



EDUCAZIONE della NICCHIA pre-metastatica mediante secrezione di fattori solubili ed esosomi e reclutamento di cellule stromali e dal midollo osseo



Microambiente e dormienza metastatica



Disseminated tumor cells
Minimal residual disease

Trombospondina
(membrana basale)
Promuove la dormienza

Dormienza metastatica

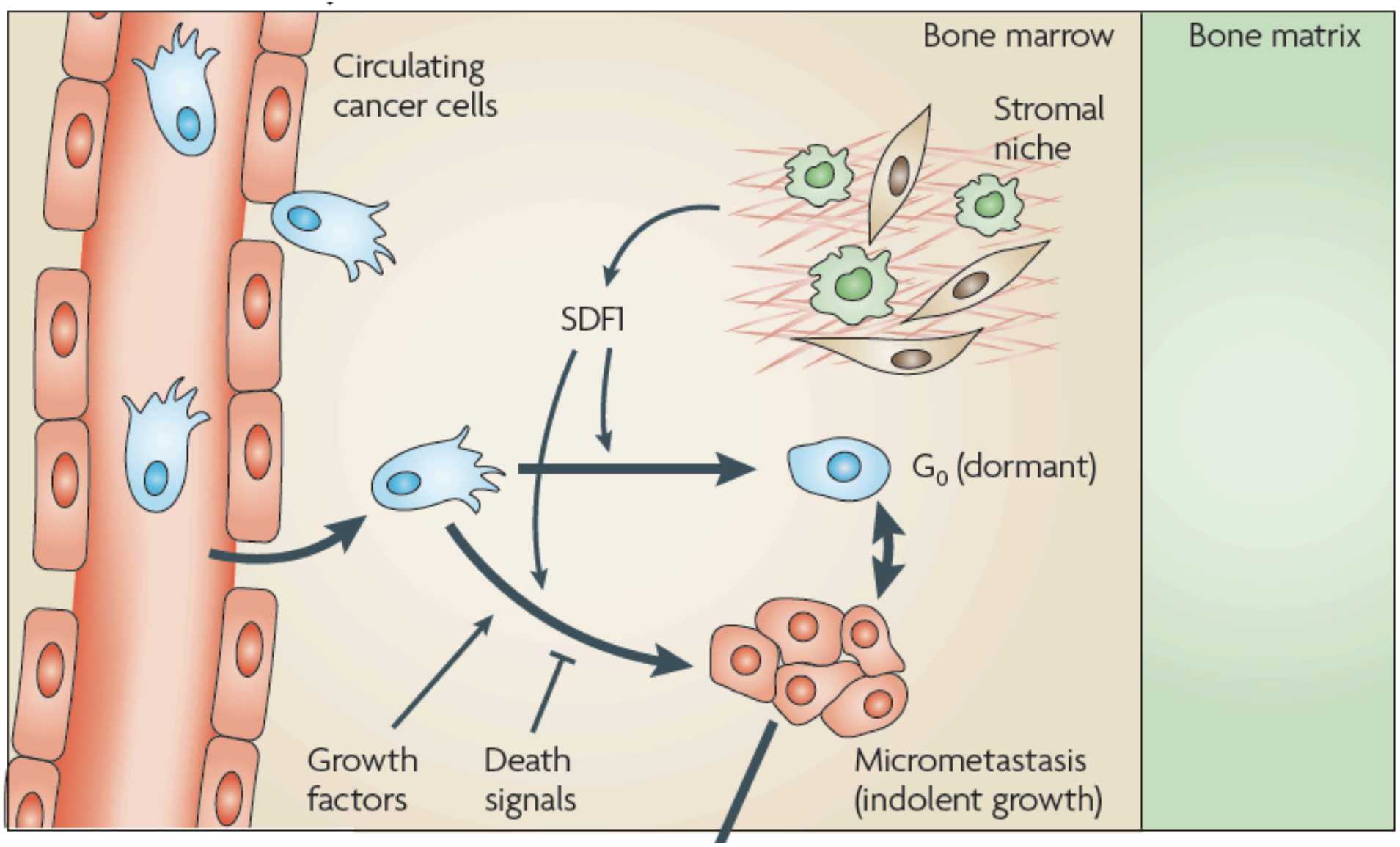
Tumour dormancy is thought to occur in two modes.
Cellular dormancy involves isolated DTCs that enter a state of proliferative quiescence. Indeed, in patient bone marrow samples most DTCs are found as quiescent single cells.
In contrast, tumour mass dormancy involves micrometastases that cease to grow due to insufficient vascularization or to constant culling by immune defences.

Tumor mass dormancy depends on the balance between proliferation and apoptosis due to :

- anti-growth signals embedded in the extracellular matrix of the normal tissue;
- tumor suppressing actions of the immune system
- maladaptation of cancer cells to surrounding stroma
- Niche competition

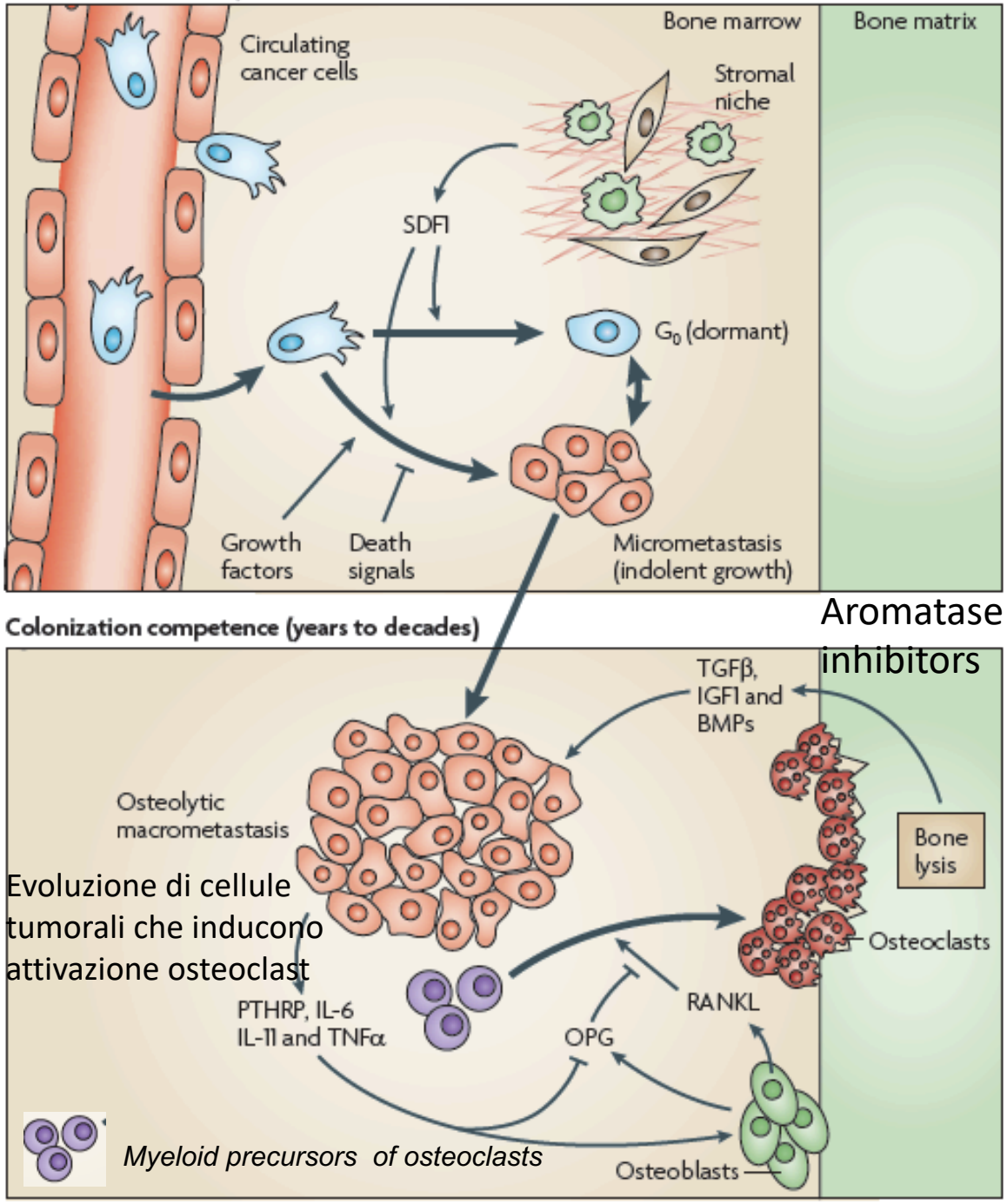
Escape from dormancy: dormant tumor cells may exit this state and resume active growth and proliferation when changes in tissue microenvironment, such as access to more nutrients (e.g. vessel formation) occur.

Dormienza metastatica



Nguyen & Massagué Nat Rev Cancer 2009

Il rimodellamento della nicchia e l'uscita dalla dormienza: metastasi ossea



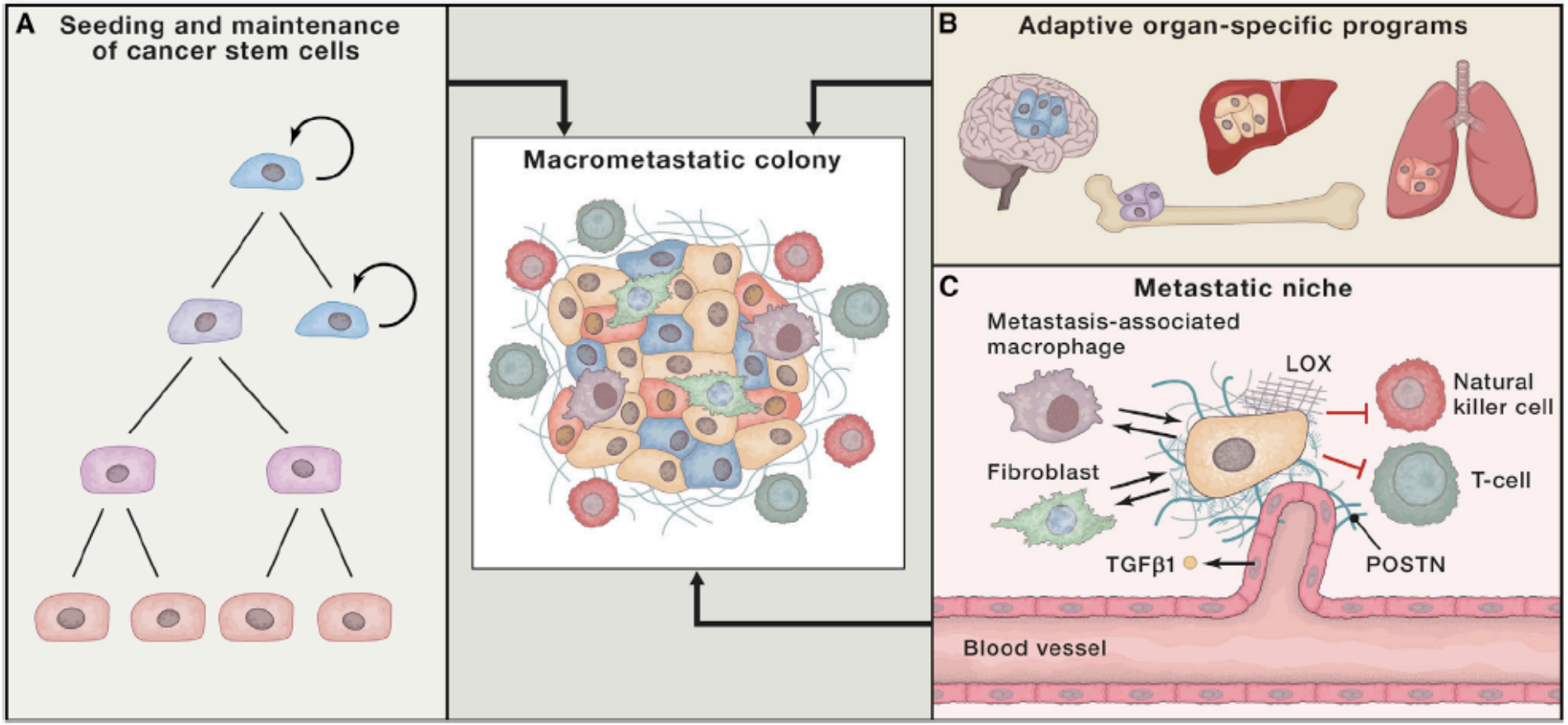
Evoluzione di cellule tumorali che inducono attivazione osteoclast

SDF1= stromal derived factor 1 (CXCL12), prodotto da cellule mesenchimali nel midollo osseo. Citochina pro-sopravvivenza.

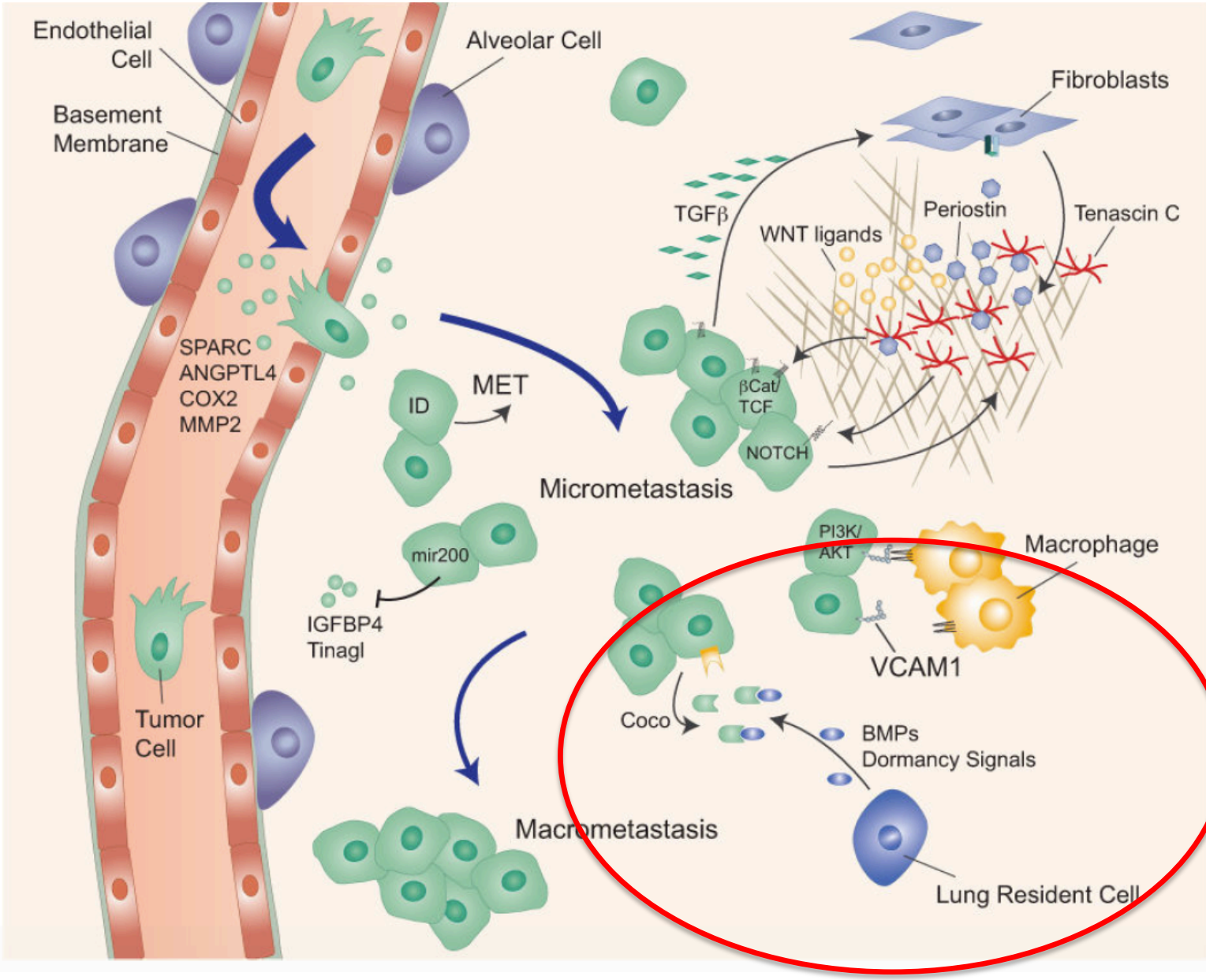
Il **rimodellamento della nicchia endostale** induce gli osteoblasti a secernere **RANKL** che **attiva gli osteoclasti** che liberano nuove citochine pro-tumorali (TGFbeta) digerendo la matrice dell'osso.

Nguyen & Massagué Nat Rev Cancer 2009

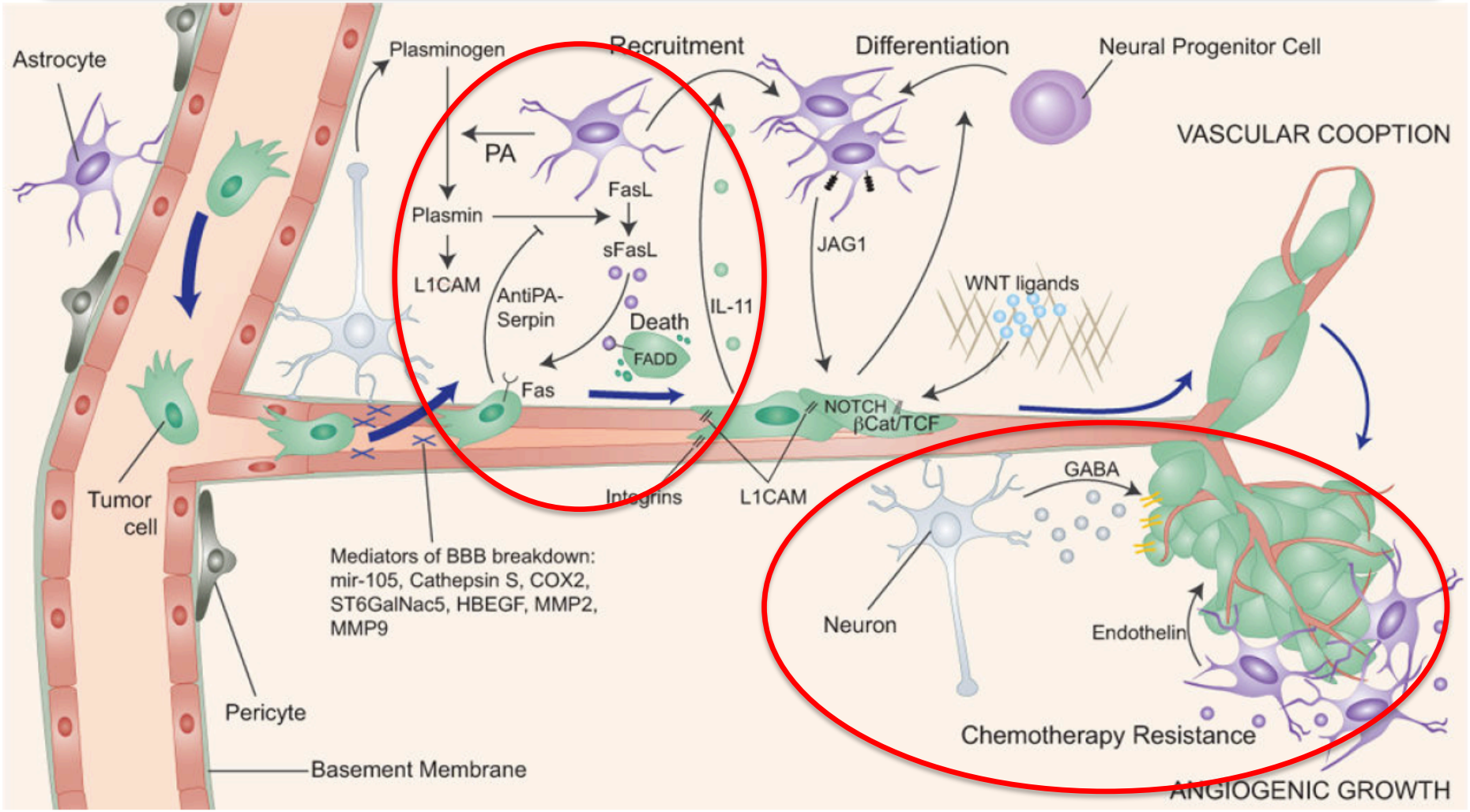
Requisiti per la colonizzazione metastatica



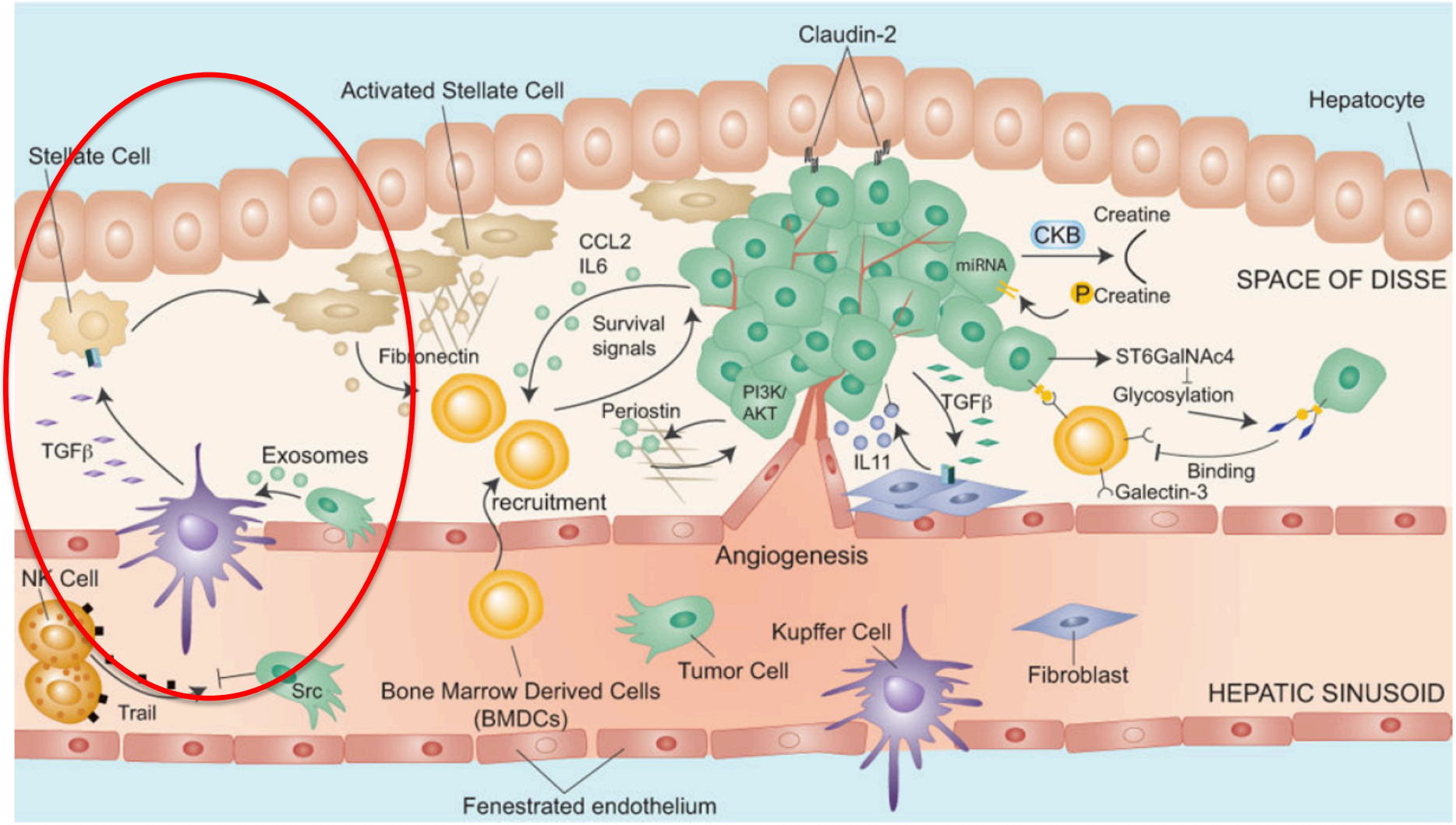
Colonizzazione metastatica del polmone



Colonizzazione metastatica del cervello



Colonizzazione metastatica del fegato



Kupffer cells = liver macrophages Stellate cells = fibrogenic liver-specific mesenchymal cells

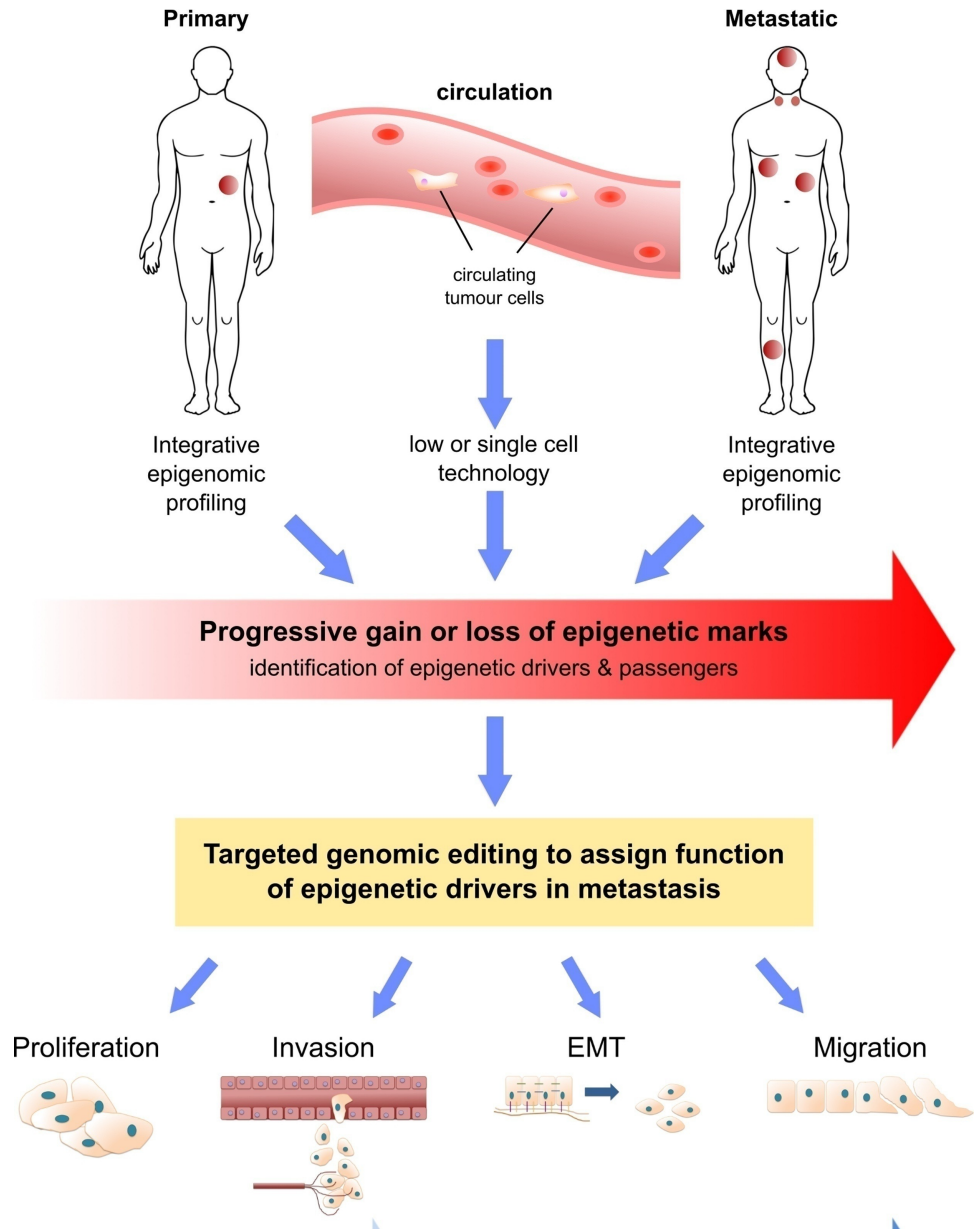
Requisiti per la colonizzazione metastatica

The ability of carcinoma cells to outgrow as lethal metastases appears to be dependent on three essential conditions.
(A) The capacity to seed and maintain a population of cancer stem cells, which are competent to re-initiate tumor growth, appears to be an initial prerequisite for metastatic growth. Dormant DTCs also exhibit key cancer stem cell attributes that probably contribute to their prolonged persistence in a quiescent state and their ability to eventually spawn a metastatic colony.

(B) Although cancer stem cells are endowed with the potential to re-initiate tumor growth, the proliferative expansion to an overt metastatic colony is dependent on the ability to contrive organ-specific colonization programs that allow these cells to thrive in a foreign tissue microenvironment. An array of organ-specific metastatic programs has been described in the literature but there is also evidence for the existence of colonization programs that confer multi-organ metastatic potential.

(C) During many stages of metastatic growth, cancer cells depend on interactions with their microenvironmental niche and cross talk with various stromal cells, including endothelial cells, fibroblasts, and cells of the innate and adaptive immune system. The ECM is also an important component of the niche and can be modified in ways that support metastatic colonization. In some cases the formation of a metastatic niche may actually precede the arrival of cancer cells, in what is referred to as a pre-metastatic niche.

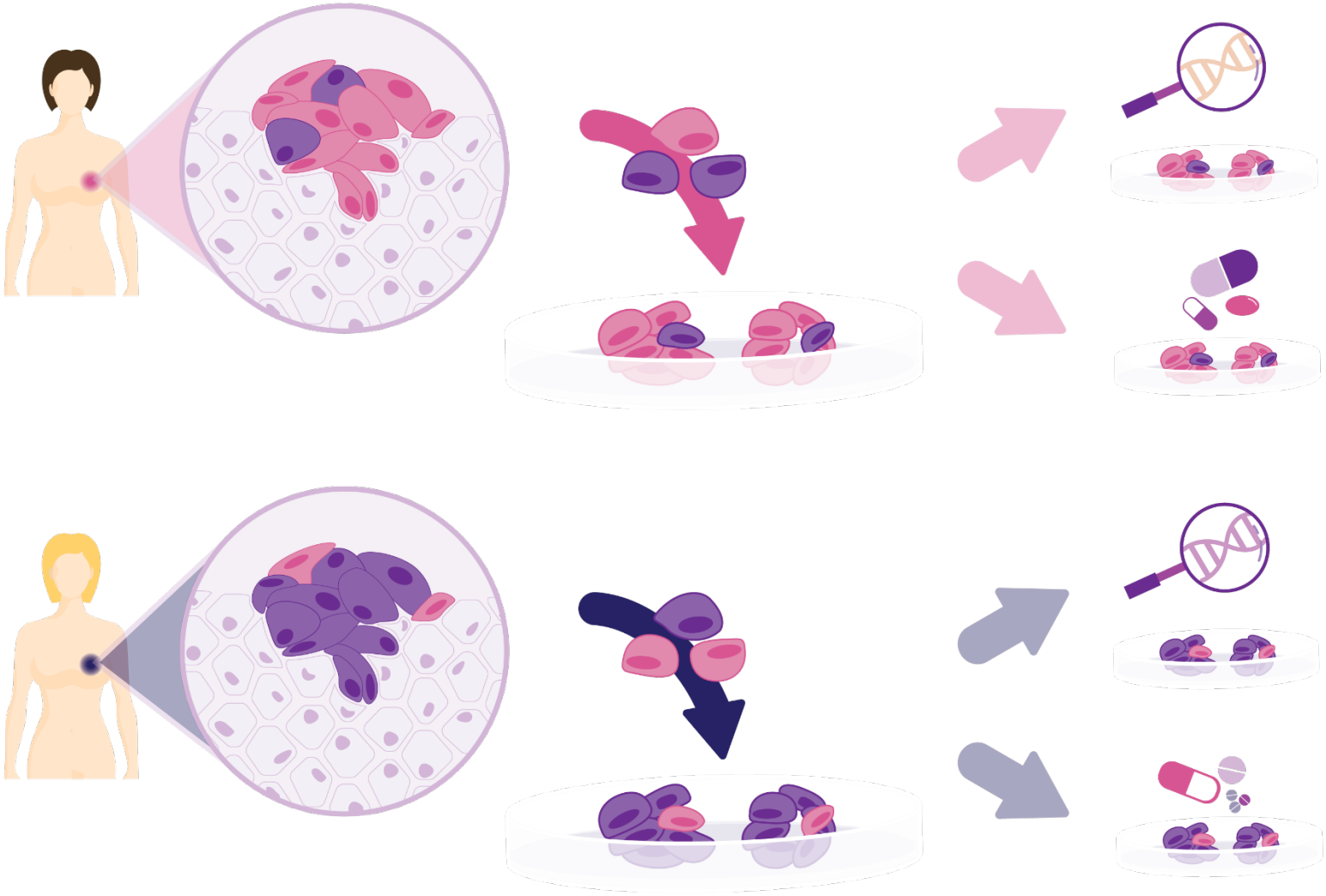
Colonizzazione metastatica: drivers genetici ed epigenetici

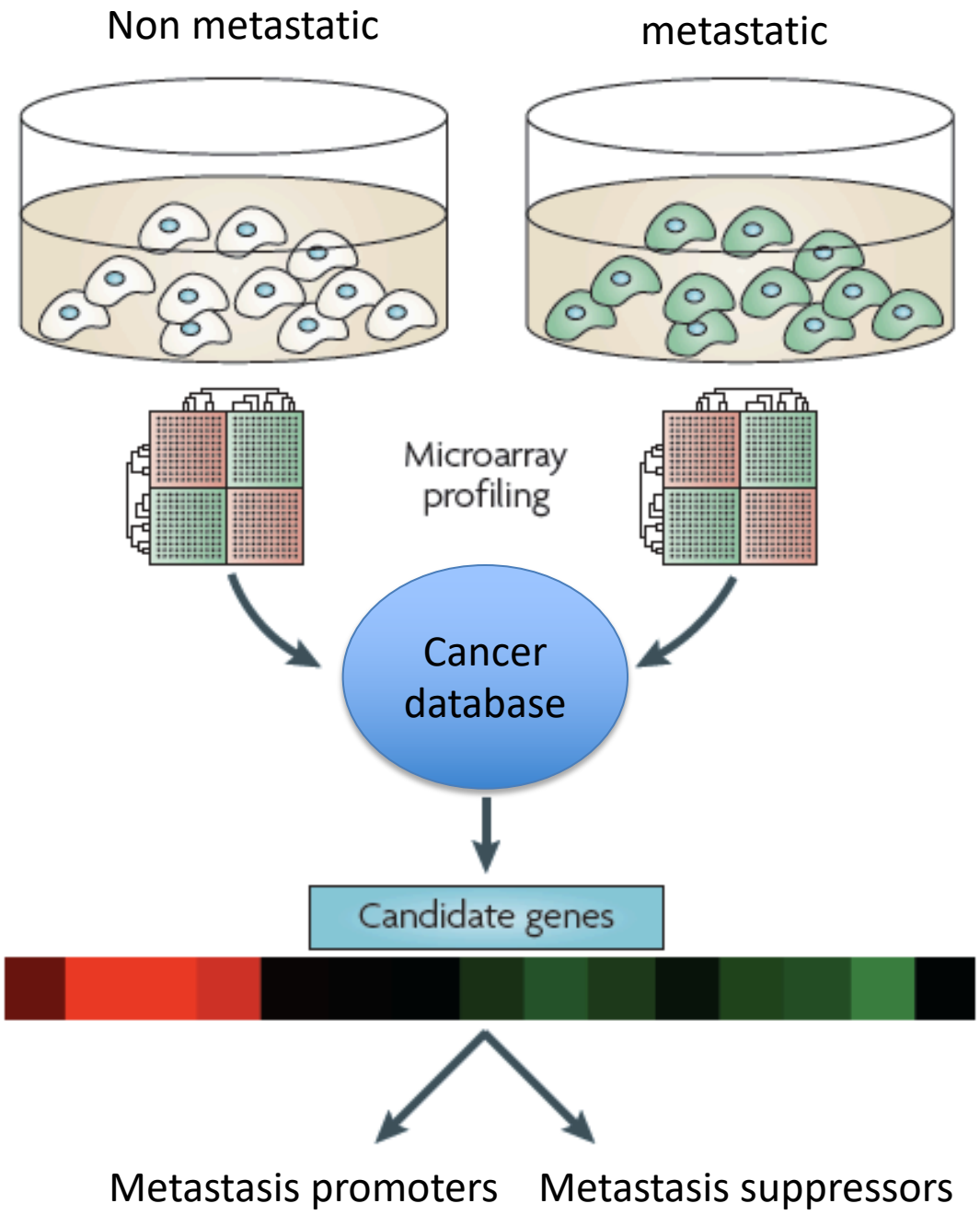


Paired **genomic/transcriptomic/epigenetic analysis** of a metastatic tumour and corresponding primary tumour from the same patient provides a **powerful approach to identify putative metastasis-drivers.** Simultaneous study of **circulating tumour cells** provides a comprehensive model for studying progressive changes during the metastatic cascade.

Functional studies and sophisticated editing experiments could then be used to understand the cellular consequences of these changes.
If the identified drivers are causal, they will have **significant translational implications** in regards to early diagnosis of cancer and potentially in developing new therapeutic strategies.

Generazione di organoidi da tumore primario e da metastasi per identificare processi driver




Non metastatic
metastatic
Microarray profiling
Cancer database
Candidate genes
Metastasis promoters
Metastasis suppressors

Geni soppressori delle metastasi

Box 1 | **The identification and characterization of metastasis suppressor genes**

Screens of cells of differing metastatic properties to identify differentially expressed candidate genes

Strategies have used screens such as chromosome transfer to screen for metastasis suppressor loci[50], differential display and subtractive hybridization techniques[30], and comparative microarray studies of cell lines exhibiting differing *in vivo* metastatic potentials[90]. Recently, workup of candidate metastasis suppressor genes and targets has been aided by available microarray expression data for many tumour histologies[108], as candidates can be quickly examined across different stage tumours for expression patterns consistent with potential metastasis suppressor function[40,90].

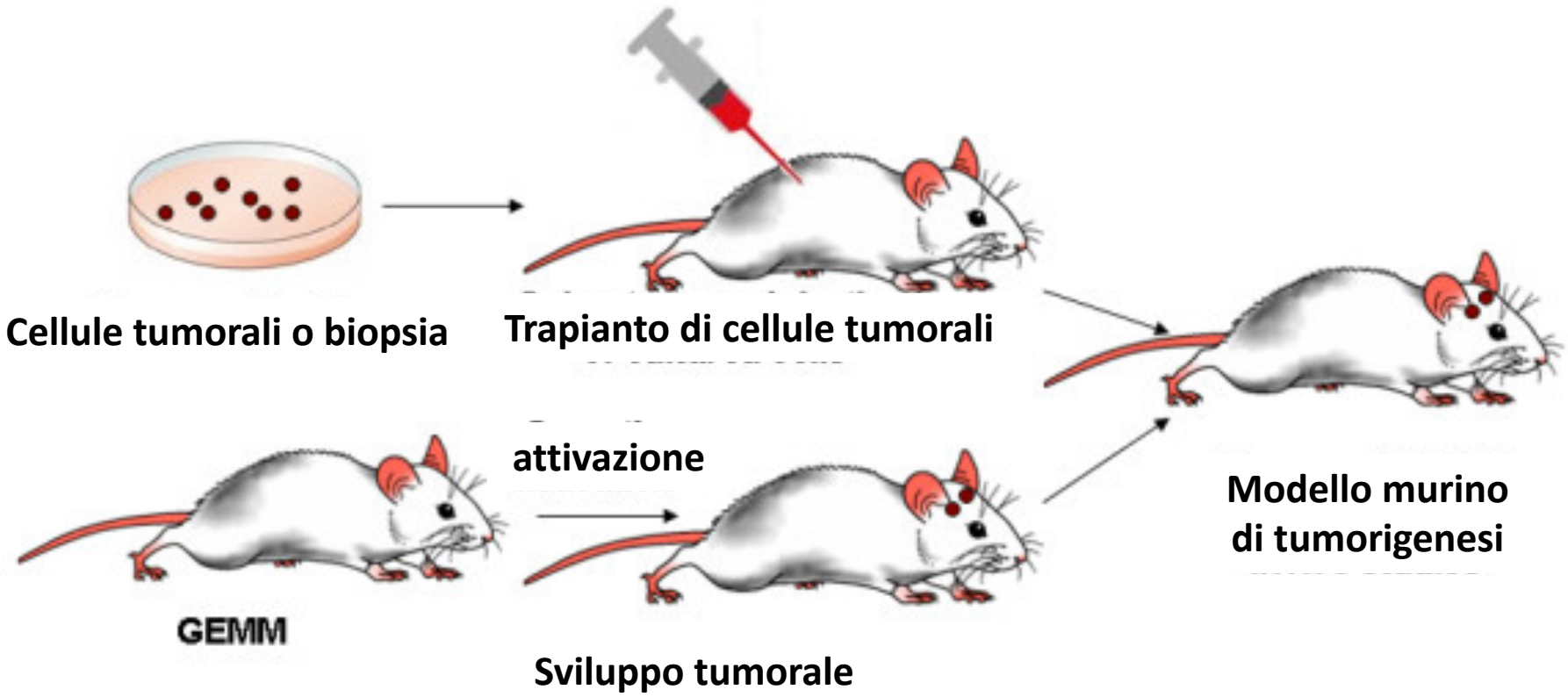
Correlation of loss of candidate gene expression or function with development of metastasis in patients

Validation of a candidate metastasis suppressor gene should include examination of its expression or function in human tumour tissues, ideally from actual metastases. However, resection of such lesions is rare for most malignancies. Instead, surrogate measures of metastatic ability are used, including whether loss of expression of the candidate in the primary tumour is associated with the development of metastasis in patient follow- up[92], clinicopathological surrogates of aggression (stage, grade and so on) or patient survival.

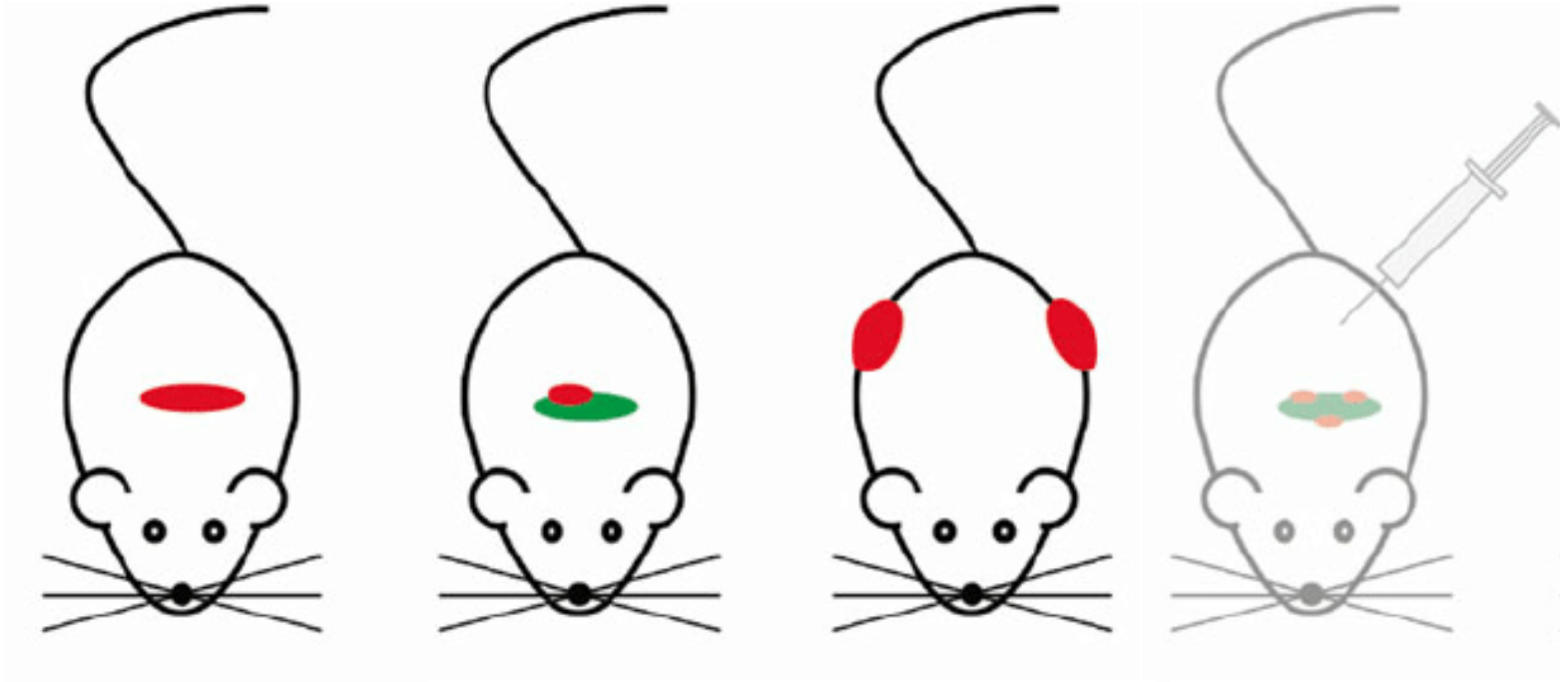
Demonstration of *in vivo* suppression of metastasis

Typically, control and derivative cells exogenously re-expressing a candidate metastasis suppressor are used to prove suppression of metastasis without hindering tumorigenesis, the *sine qua non* for defining a metastasis suppressor protein. These experiments generally use tumour-derived cell lines introduced into rodents[10]. Spontaneous metastasis assays examine cells in an orthotopic xenograft[109] or at subcutaneous or other sites in a heterotopic xenograft[110]. These evaluate the majority of the steps in the metastatic process, but not all tumour types have xenograft models amenable to this assay. Experimental metastasis assays involve injection of tumour cells directly into the arterial or venous circulation[111], examining only the latter portion of the metastatic process, but can be faster and more reproducible than spontaneous metastasis assays. Either way, effects on primary tumour growth must be ruled out by subcutaneous or orthotopic xenograft assays as well.

Saggi di metastaticità in vivo



Modelli murini di tumorigenesi



Genetic Engineered Mouse (GEM)

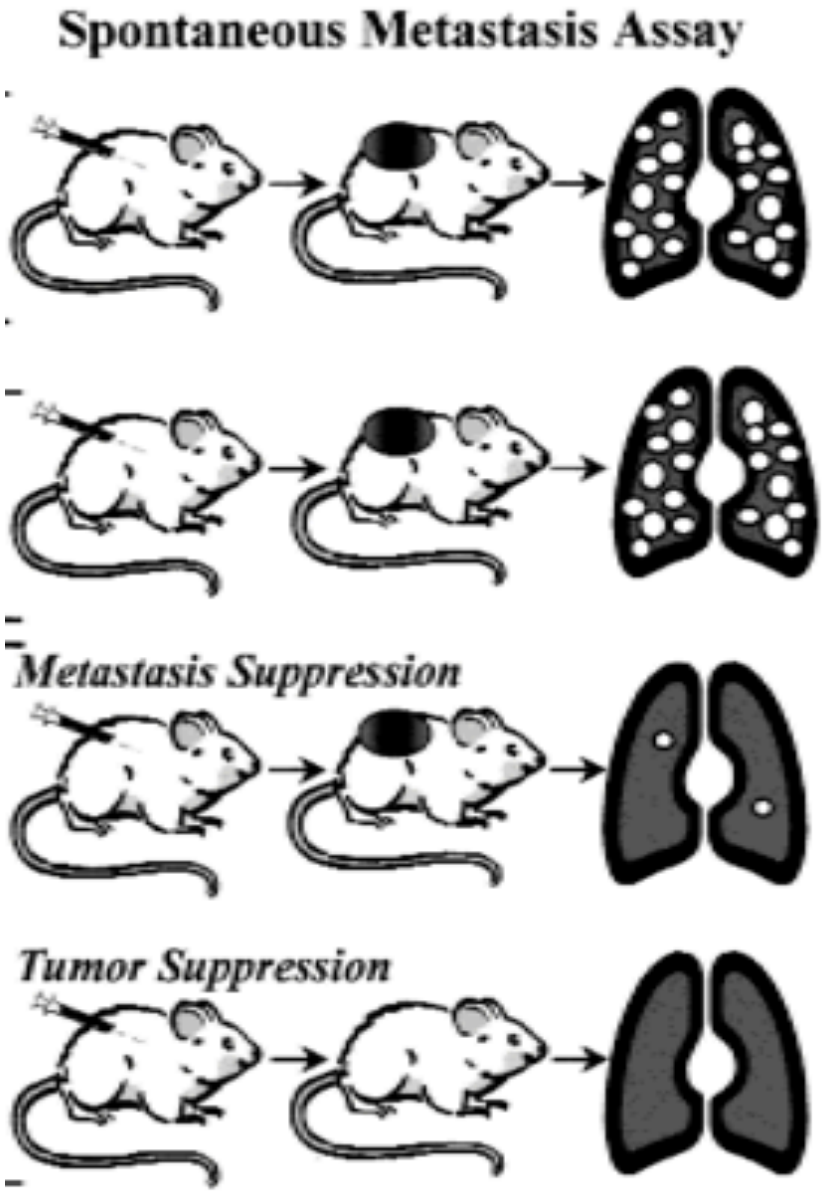
Trapianto ortotopico o eterotopico

spontaneous metastasis

Iniezone iv o intracardiaca

Experimental metastasis

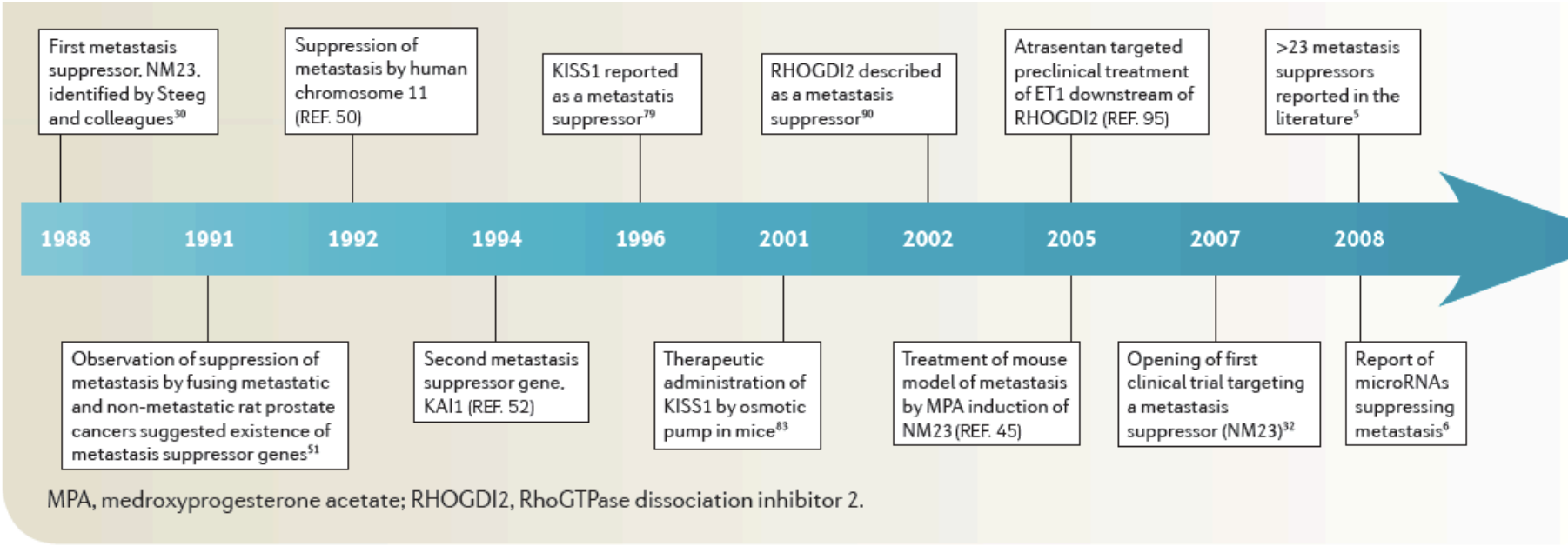
Validation of metastasis suppressor genes



Geni soppressori delle metastasi

Istidina chinasi, inibisce Ras signaling e espressione di geni pro-metastatici

Peptidi secreti che mantengono la dormienza



MPA, medroxyprogesterone acetate; RHOGDI2, RhoGTPase dissociation inhibitor 2.

Proteina di membrana inibisce EGFR

Geni soppressori delle metastasi