Tumor Metastasis

Key Steps in Tumor Metastasis

1. Separation of tumor cells from the primary tumor mass.

Tissue Invasion

2. Invasion of tumor cells through surrounding tissue.
   - Proteolytic destruction of the basement membrane and extracellular matrix (ECM).
3. Migration towards blood vessels.

Intravasation

4. Entry of tumor cells into a blood vessel and their activation by platelet-activating factor.
5. Increased expression of P-selectin and the formation of tumor cell-platelet aggregates.

Circulation

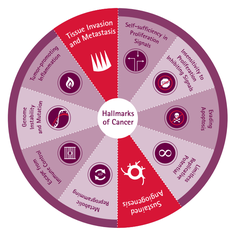
6. Tumor cells reach distant sites via circulation in the blood vessel.

Extravasation

7. Attachment of activated tumor cells to the vessel wall, glycosylation of integrin, CD44, and cadherin, and exit from blood vessel.
8. Tumor cell divides; this is the beginning of a secondary growth.

Sustained Angiogenesis

9. Tumor-associated macrophages secrete growth factors.
10. Hypoxia in a growing tumor mass results in the production of HIF, which stimulates the production and release of growth factors and proteolytic enzymes.
    - Angiogenic growth factors bind to specific receptors located on the endothelial cells and stimulate their proliferation and migration.
11. Endothelial cells begin to proliferate and migrate towards the tumor mass.
    - MMPs degrade the basement membrane and extracellular matrix. Adhesion molecules (such as integrins) help the forming blood vessel to grow towards the tumor.
12. New blood supply is established.
    - As the vessel extends, the tissue is remolded around the vessel and the sprouting endothelial cells roll up to form a blood vessel tube.



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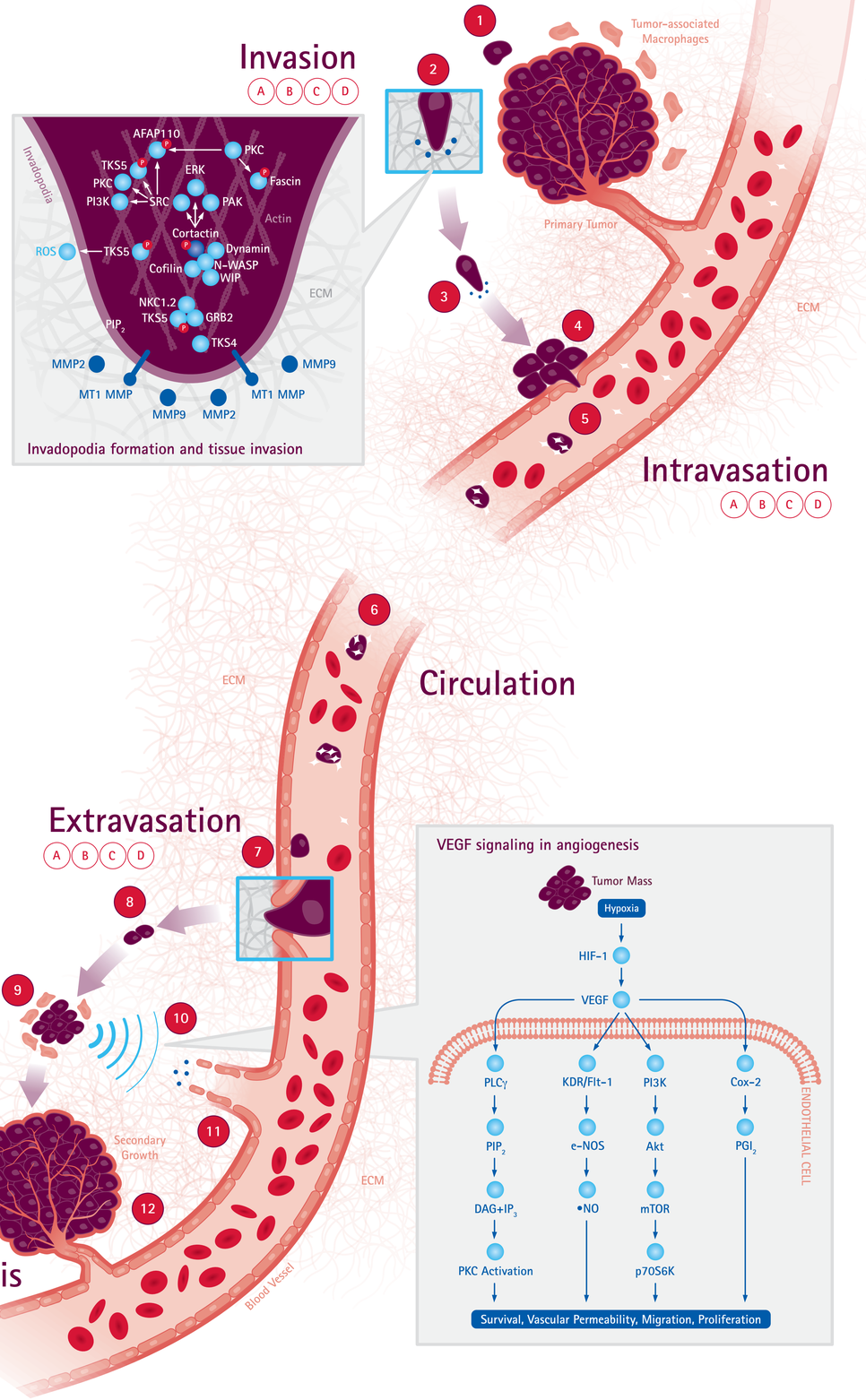
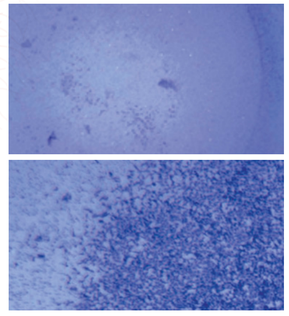


Techniques & Assays

A Boyden Chamber

TO STUDY	USE
Cell Migration	QCM™ ECMatrix Cell Invasion Assay,
Cell Invasion	Colorimetric (Cat. No. ECM550)

The classic Boyden Chamber system places cells in a extracellular matrix protein-coated porous membrane insert over a chemoattractant-containing well. Invading cells migrate through the pores, are stained and measured.



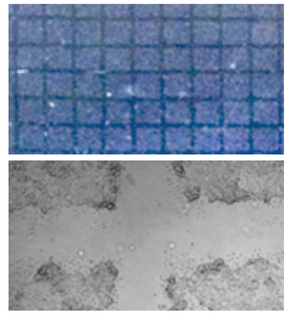
QCM™ ECMatrix™ Cell Invasion Assay, colorimetric was used to demonstrate lack of invasion by non-invasive NIH3T3 cells (top panel), and active invasion by HT-1080 cells (bottom panel).

B Scratch Assay

TO STUDY	USE
Cell Migration	Cell Comb™ Scratch Assay
Wound Healing	(Cat. No. 17-10191)

The basic scratch assay creates physical wounds in a cell monolayer for observing cell migration.

The Cell Comb™ Scratch Assay has been optimized to apply a high density field of scratches to create a high proportion of migrating cells to quiescent monolayer cells, that permits sensitive detection of the biochemical events in the migrating cell population.



Cell Comb™ Scratch Assay was used to create cell monolayers with multiple wounds applied two directions with the Cell Comb™ was viewed at 2X magnification (top) and 10X (phase contrast image, bottom).

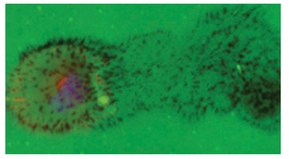
For a complete listing of Cancer Products, visit:

www.merckmillipore.com/cancer

C ECM Degradation

TO STUDY	USE
Cell Migration	QCM™ Gelatin Invadopodia Assays (Green
Invasion	& Red) (Cat. Nos. ECM670 & ECM671)
Intravasation	

The most widely published method for measuring extracellular matrix degradation due to "invadopodia" or "podosomes" involves plating cells over a culture surface coated with a thin layer of fluorescently labeled matrix, and visualizing regions where the cell has degraded the matrix to create an area devoid of fluorescence.

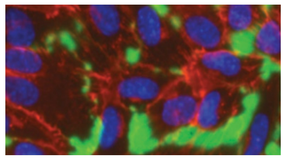


QCM™ Gelatin Invadopodia Assay (Green) was used to study cell migration across a fluorescently-conjugated matrix. Dark spots are evidence of invadopodia.

D Vascular Permeability Assay

TO STUDY	USE
Extravasation	*In Vitro* Vascular Permeability Assays (96-well, 24-well & Imaging Formats) (Cat. Nos. ECM642, ECM644 & 17-10398)

The endothelial cell lining inside the vasculature defines a semi-permeable barrier between the blood and the interstitial spaces of the body. *In vitro* vascular permeability studies involve an intact, confluent endothelial cell monolayer cultured on semi-permeable membranes or clear chamber slides. Disruptions of the barrier integrity can be measured by multi-well plate-based assays or visual detection.

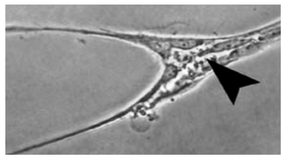


In Vitro Vascular Permeability Imaging Assay was used to visualize changes in microvascular hyperpermeability.

E Tube Formation Assay

TO STUDY	USE
Angiogenesis	*In Vitro* Angiogenesis Assay (Cat. No. ECM625)
	Fibrin *In Vitro* Angiogenesis Assay (Cat. No. ECM630)
	Millicell® µ-Angiogenesis Inhibition Assay Kit (Cat. No. MMA125)
	Millicell® µ-Angiogenesis Activation Assay Kit (Cat. No. MMA130)

The tube formation assay has been typically employed to demonstrate the angiogenic activity of vascular endothelial cells *in vitro*. This assay can serve as a powerful method to screen the vascular potential of a variety of cell types including vascular cells, tumor cells, as well as other cells.



Fibrin *In Vitro* Angiogenesis Assay was used to study tube formation in HUVEC (32x magnification).

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