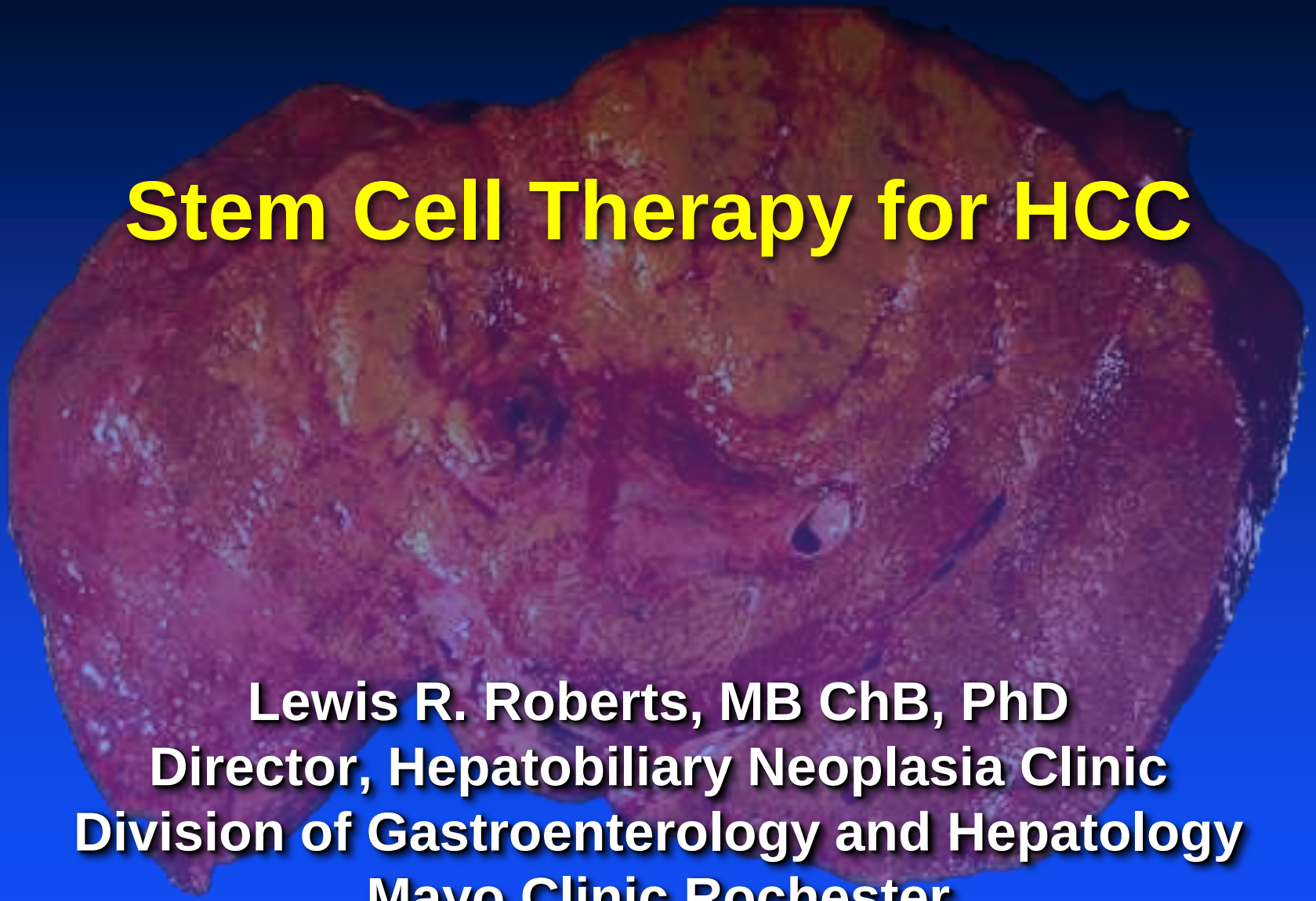
Stem Cell Therapy for HCC



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Expanded Hallmarks of Cancer

Independence from Growth Factors

Limitless replication

Deregulation of Metabolism

Genomic Instability

Angiogenesis

Resistance to Apoptosis

Immune Escape

Inflammation

Metastasis & Invasion

Evasion of Tumor Suppression

Hanahan and Weinberg: Cell 144:646, 2011

The Tumor Microenvironment in Liver Cancer : Key Concepts

- The proliferative cancer platform is critical, but only part of full tumorigenesis
- Multiple growth factors and cytokines regulate extracellular matrix production
- Extracellular matrix signals contribute to tumor progression and metastasis
- The tumor microenvironment provides potential new targets for HCC therapy

The Hallmarks of Cancer: The Tumor Microenvironment

Tumors are not, as was previously thought: "nothing more than a collection of relatively homogeneous cancer cells, whose entire biology can be understood by elucidating the cell autonomous properties of these cells."

Hanahan and Weinberg: Cell 144:646-674, 2011

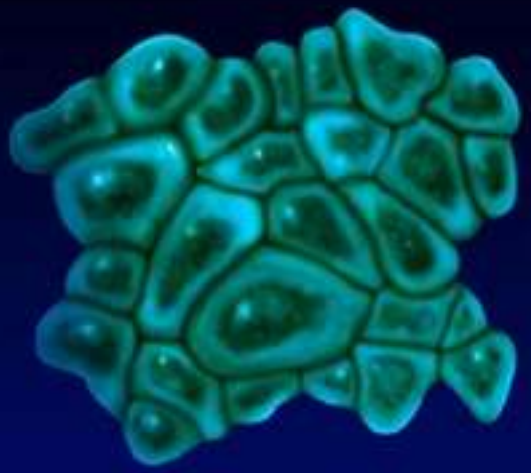
The Hallmarks of Cancer: The Tumor Microenvironment

Rather, 'tumors have increasingly been recognized as organs whose complexity approaches and may even exceed that of normal healthy tissues. When viewed from this perspective, the biology of a tumor can only be understood by studying the individual specialized cell types within it as well as the "tumor microenvironment" that they construct during the course of multistep tumorigenesis.'

Hanahan and Weinberg: Cell 144:646-674, 2011

The Tumor Microenvironment Influences Primary and Secondary Oncogenic Events

Primary event

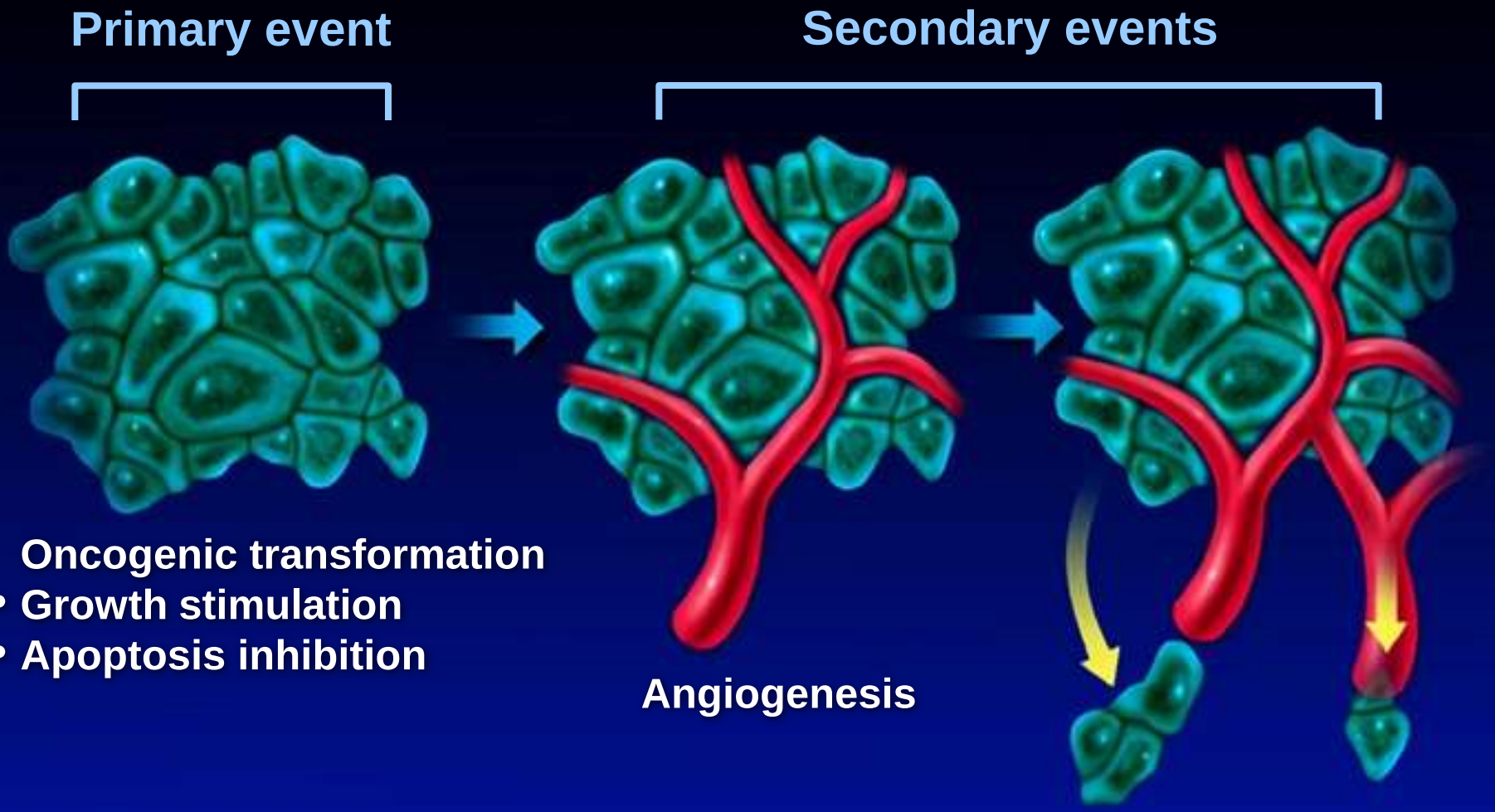


Oncogenic transformation

- **Growth stimulation**
- **Apoptosis inhibition**

Hoshida Y, NEJM 2008; Roessler S, Cancer Research 2010

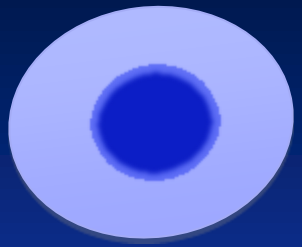
The Tumor Microenvironment Influences Primary and Secondary Oncogenic Events



Hoshida Y, NEJM 2008; Roessler S, Cancer Research 2010

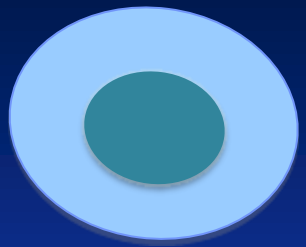
Cells Participating in Liver Tumor Microenvironment

Target Cells



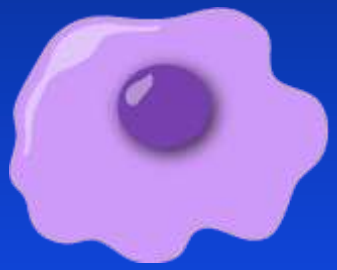
Cholangiocyte

Liver Stem Cell

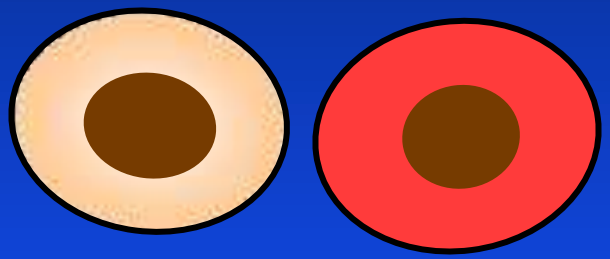


Hepatocyte

Immune/ Inflammatory Cells



Kupffer Cells/
Tumor Associated Macrophages



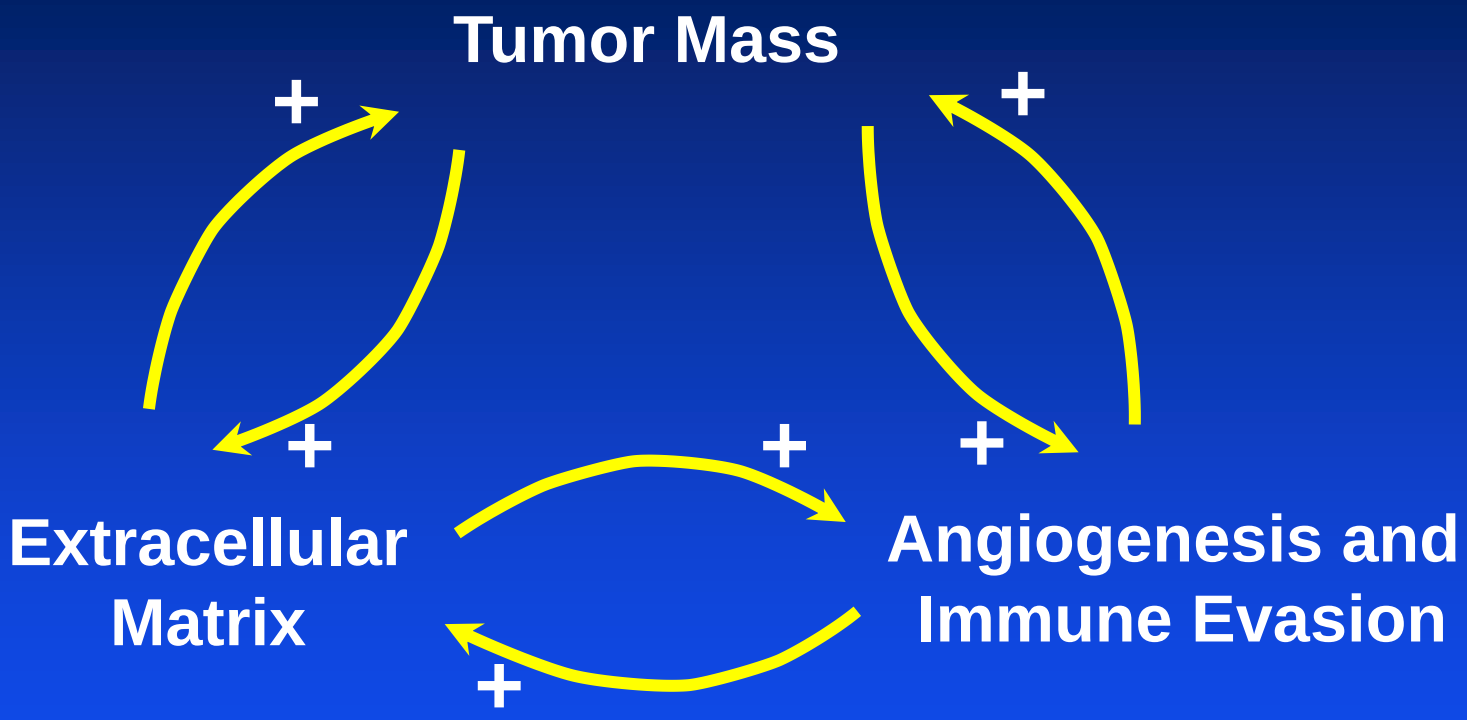
B and T Lymphocytes

Angiogenic/ Fibrogenic Cells

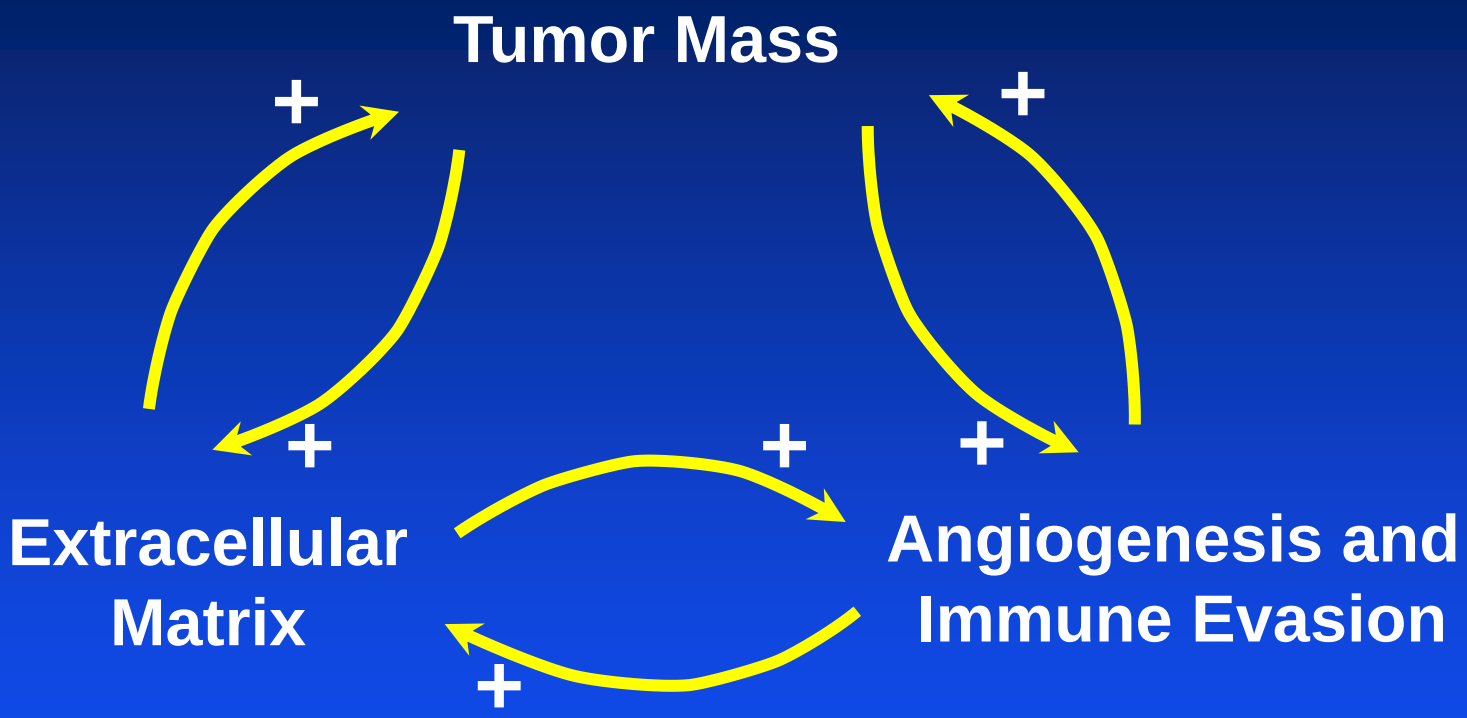
Endothelial Cells

Stellate Cells/Fibroblasts

The Growing Tumor Mass, the Extracellular Matrix, and Angiogenic and Immune Evasion Pathways Regulate Each Other by Positive Feedback Loops



The Growing Tumor Mass, the Extracellular Matrix, and Angiogenic and Immune Evasion Pathways Regulate Each Other by Positive Feedback Loops



This results in a tumor running amok!

Mesenchymal Stromal Cells (MSCs)

- Adherence to plastic in culture
- Self renewal properties
- Low immunogenicity
- Trophic activity
- Promotion of vascularization
- Multipotent differentiation potential
- Migrate and home towards tumors
- Characteristic protein expression profile
- Express CD105, CD73, and CD90
- Lack CD45, CD34, CD14/11b, CD79α/CD19, and HLA class II
- Can differentiate in vitro into osteoblasts, adipocytes, chondroblasts, cardiomyocytes, vascular endothelial cells, neurons, hepatocytes and/or other epithelial cells

Sources of MSCs

- Bone marrow
- Adipose tissue
- Peripheral blood
- Extraembryonic tissue after birth
  - Placenta
  - Amnion
  - Umbilical cord

Migratory Capability of MSCs

- Powerful component of human body repair
- In physiological conditions low numbers circulate through peripheral blood; mainly reside in BM niche
- Endocrine hormones released in response to injury mobilize MSCs into the bloodstream and migration to injured sites to promote tissue regeneration
- Engraft in injured or inflamed tissues

Microenvironmental Effects of MSCs

- The tumor microenvironment is characterized by increased production of inflammatory mediators and chemoattractants
- MSCs show increased motility towards tumorigenic sites (the tumor behaves as an unresolved wound that attracts MSCs)
- This capability has been exploited in using MSCs for delivery of interferon-β and other cytokines

Receptors Mediating MSC Transmigration

- PDGFR
- VEGFR1/2
- IGF1R
- CCR6
- CXCR1
- CXCR4

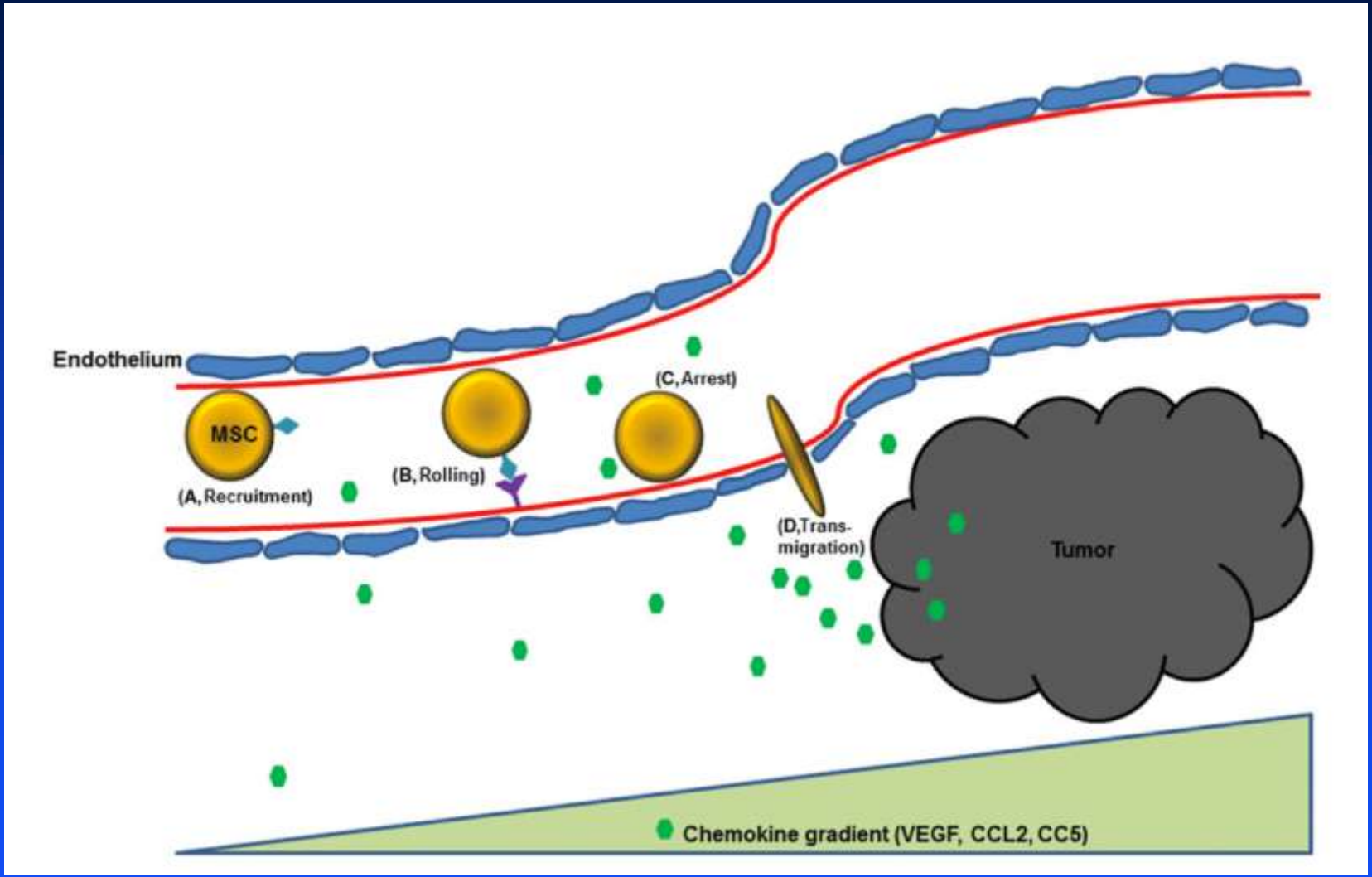
Factors Mediating MSC Transmigration

- VEGF
- PDGF-BB
- TGF-β
- M-CSF
- MCP-1
- IL-8
- TNF-α
- IL-1β
- IL-6
- SDF-1
- HGF

Mechanisms of MSC Migration into HCC

- Integration
  - Retraction of endothelial cells
  - Spreading and incorporation of MSCs into the endothelial monolayer
  - Relocalization of endothelial cells on top of MSCs
- Paracellular diapedesis
- Transcellular diapedesis

Mechanisms of MSC Migration into HCC



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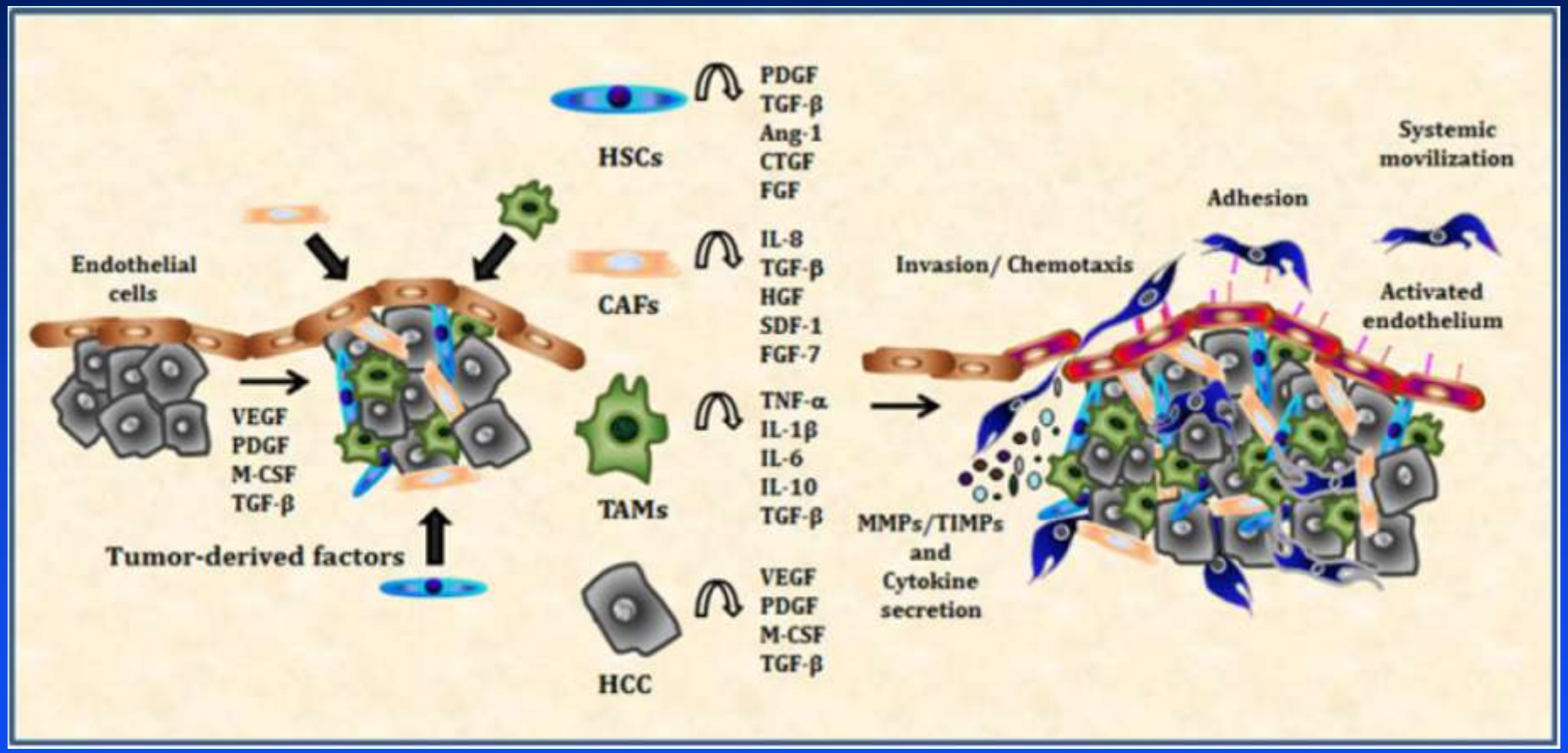
Recruitment of MSCs into HCC



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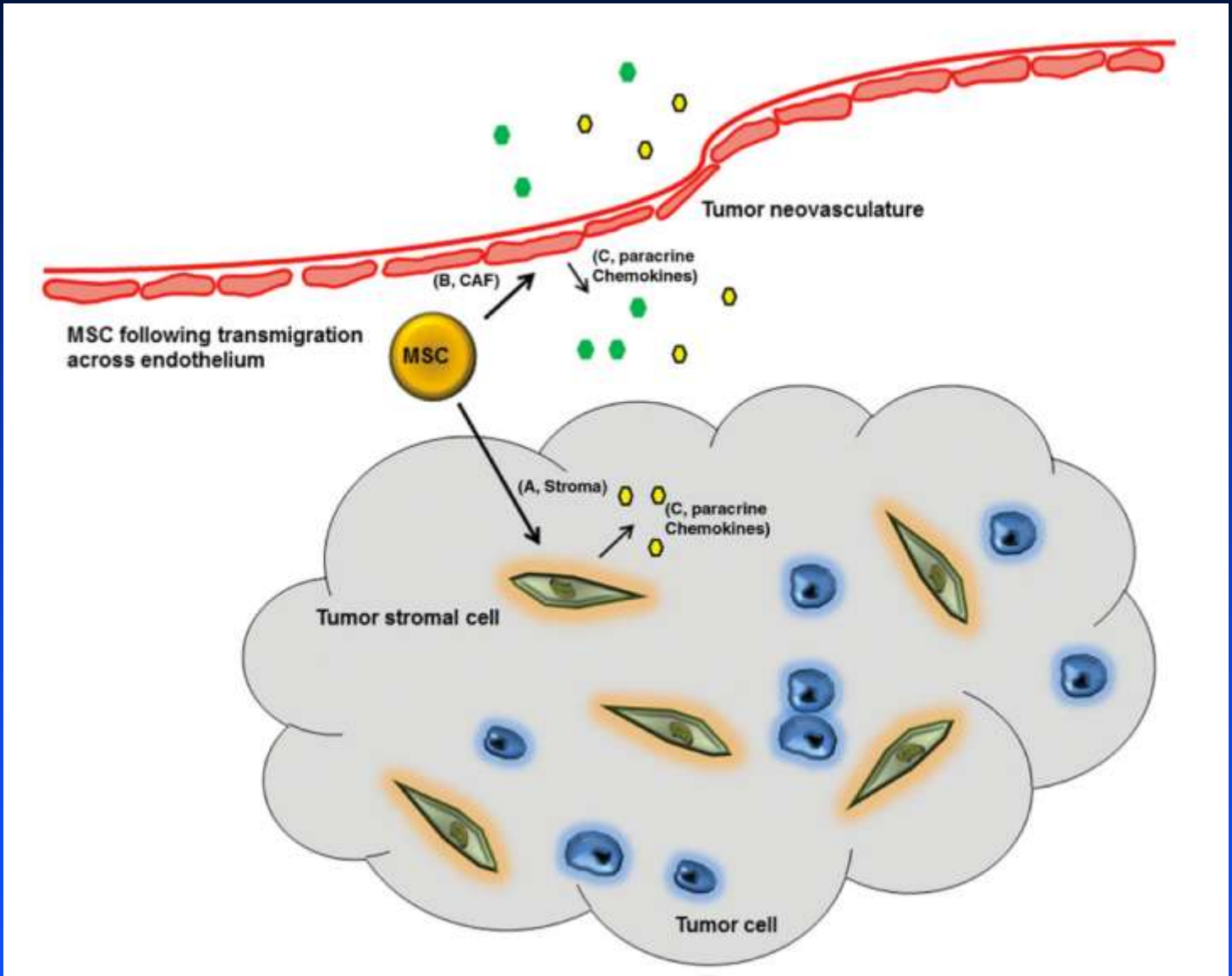
Role of MSCs in Tumor Progression



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Matrix Components of the HCC Microenvironment Involved in MSC Migration

- Collagen IV
- Fibronectin
- Glycosaminoglycans
- MMP2

Sheng-Di Wu 2012

How Do MSCs Affect HCC Growth and Progression? Mixed Results

Table 1. Studies on the effect of applying non-genetically modified MSCs in experimental HCC models

MSCs characteristics		Animal Model	Biological effect	Reference
Source	BALB/c BM-derived MSCs	i.p. injection of MSCs (5×10^5) in BALB/c mice with s.c. tumour (BNL cells)	Without effect on tumour development	X. Chen et. al. 2006 (36)
# Passage	6 to 9			
Surface markers	ND			
Source	BALB/c BM-derived MSCs	i.v. injection of MSCs (initial:2×10^6; then 1×10^6 every 5 days/20 days) in nude BALB/c mice with s.c. tumour (Hca cells)	Without effect on tumour growth	X. Chen et. al. 2008 (40)
# Passage	6 to 9			
Surface markers	$CD44^+$, $CD105^+$ and $CD73^+$			
Source	Dermis, foetal human: Z3 MSCs, immortalized cell line	s.c. co-injection of MSCs (1×10^7) with H7402 tumour cells in SCID mice	Inhibition of tumour growth	L. Qiao et. al. 2008 (41)
# Passage	ND			
Surface markers	$CD29^+$,$CD44^+$,$CD105^+$, $CD166^+$, $CD31^-$, $CD45^-$, hTERT+, $CD34^-$, vWF and HLA-DR^-			
Source	Mouse BM-derived MSCs	i.p. injection of MSCs (2×10^5/0, 3 and 10 days after tumour induction) in BALB/c mice with ascitogenous hepatoma (H22 cells).	Inhibition of tumour volume and ascites formation	Y. Lu et. al. 2008 (134)
# Passage	2 to 4			
Surface markers	$CD73^+$, $CD90^+$, HLA-DR^-			
Source	Human BM-derived MSCs	i.v. injection of MSCs (5×10^5/3 × week/4 weeks) in nude BALB/c mice with s.c. tumour (MHCC97-H cells)	Promotion of tumour growth. Inhibition of metastasis development	G. Li et. al. 2010 (135)
# Passage	5 to 8			
Surface markers	$CD44^+$ and $CD90^+$			
Source	Human BM-derived MSCs	i.v. injection of MSCs (1×10^6) in nude BALB/c mice with i.h. tumour (MHCC97-H cells)	Without effect on tumour growth	Y. Gao et. al. 2010 (94)
# Passage	3 to 4			
Surface markers	$CD105^+$,$CD29^+$,$CD90^+$, $CD45^-$, $CD34^-$ and $CD14^-$			
Source	Human BM-derived MSCs	i.v. injection of MSCs (5×10^5) in nude BALB/c mice with s.c. tumour (HuH7 cells)	Without effect on tumour growth	M. Garcia et. al. 2011 (92)
# Passage	4 to 6			
Surface markers	CD44+, CD49e+, CD73+, CD90+, $CD105^+$, $CD166^+$, $CD31^-$, $CD34^-$, $CD45^-$, $CD14^-$ and $CD79^-$			
Source	C57BL/6 $p53^{-/-}$ BM-derived MSCs	i.v. injection of MSCs (5×10^5/week/3 weeks) in nude BALB/c mice with i.h. tumour (HuH7 cells)	Promotion of tumour growth and angiogenesis	H. Niess et. al. 2011 (93)
# Passage	ND			
Surface markers	$CD73^+$, $CD105^+$,$CD34^-$, $CD14^-$, $CD45^-$ and HLA-DR^-			
Source	Human BM-derived MSCs	i.t. injection of CM-MSCs (100 μg/2 × week/3 weeks) in nude SCID mice with s.c. tumour (HepG2 cells)	Enhancement of tumour growth	C. Cavalliari et. al. 2012 (136)
# Passage	6			
Surface markers	$CD105^+$, $CD73^+$, $CD90^+$, $CD166^+$, $CD44^+$, $CD45^-$, $CD14^-$, $CD34^-$, $CD80^-$,$CD86^-$, $CD40^-$, $CD31^-$ and vWF$^-$			

BM, bone marrow; CM, conditioned media; i.h., intrahepatic; i.p., intraperitoneal; i.t., intratumoural; i.v., intravenous; ND, no data; s.c., subcutaneou

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How Do MSCs Affect HCC Growth and Progression? Mixed Results

- In some models MSCs suppress tumor growth, but
- In other models MSCs enhance tumor growth, and
- In many models there is no effect
- Need for standardization of isolation, characterization and expansion
- Need for primate and human testing
- Consequently at this point treatment with MSCs alone cannot be recommended

Strategies for Genetic Modificaion of MSCs in HCC Therapy

Table 2. Studies of applying genetically modified MSCs in experimental HCC models

MSCs characteristics		Animal model	Biological effect	Reference
Source # *Passage* *Surface markers*	BALB/c BM-derived MSCs 6 to 9 ND	i.p. injection of MSCs adenovirally engineered to secrete interleukin-12 (5×10^5) one week before of s.c. tumour implantation (BNL cells)	Prevention of tumour establishment	X. Chen et. al. 2006 (36)
Source # *Passage* *Surface markers*	BALB/c BM-derived MSCs 6 to 9 $CD44^+$, $CD105^+$, and $CD73^+$	Nude BALB/c mice with s.c. tumour (Hca cells). i.v. injection of MSCs adenovirally engineered to secrete interleukin-12 (initial: 2×10^6; then 1×10^6 every 5 days/20 days)	Suppression of tumour growth and antimetastatic effect	X. Chen et. al. 2008 (40)
Source # *Passage* *Surface markers*	Human BM-derived MSCs 3 to 4 $CD105^+$, $CD29^+$, $CD90^+$, $CD45^-$, $CD34^-$ and $CD14^-$	Nude BALB/c mice with i.h. tumour (MHCC97-H cells). i.v. injection of MSCs engineered to express hPEDF by lentiviral transduction (1×10^6)	Antiangiogenesis. Inhibition of tumour growth. Increased animal survival	Y. Gao et. al. 2010 (94)
Source # *Passage* *Surface markers*	C57BL/6 p53−/− BM-derived MSCs ND $CD73^+$, $CD105^+$, $CD34^-$, $CD14^-$, $CD45^-$ and HLA-DR^-	Nude BALB/c mice with i.h. tumour (HuH7 cells). i.v. injection of MSCs expressing HSC-TK gene under the promoter/enhancer for CCL5 or Tie2 (5×10^5/week/3 weeks) + GCV	Inhibition of tumour growth	H. Niess et. al. 2011 (93)
Source # *Passage* *Surface markers*	Human BM-derived MSCs (immortalized cell line) ND $CD73^+$, $CD105^+$, $CD34^-$, $CD14^-$, $CD45^-$ and HLA-DR^-	CD1 nu/nu mice with s.c. tumour (HuH7 cells). Three cycles of i.v. injection of MSCs expressing NIS (5×10^5) followed by ^{131}I application	Inhibition of tumour growth and reduction of tumour vessel density	K. Knoop et. al. 2011 (146)
Source # *Passage* *Surface markers*	Human BM-derived MSCs (UE7T-13, immortalized cell line) ND ND	Nude BALB/c mice with s.c. tumour (MHCC97-H cells). i.v. injection of MSCs expressing TRAIL (1×10^6) + i.p cisplatin (1.5 mg/kg, every 3 days/21 days)	Inhibition of tumour growth and reduction of tumour vessel density	B. Zhang et. al. 2012 (145)

BM, bone marrow; i.h., intrahepatic; i.p., intraperitoneal; i.t., intratumoural; i.v., intravenous; ND, no data; s.c., subcutaneous; GCV, ganciclovir.

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MSCs and Gene Therapy Strategies

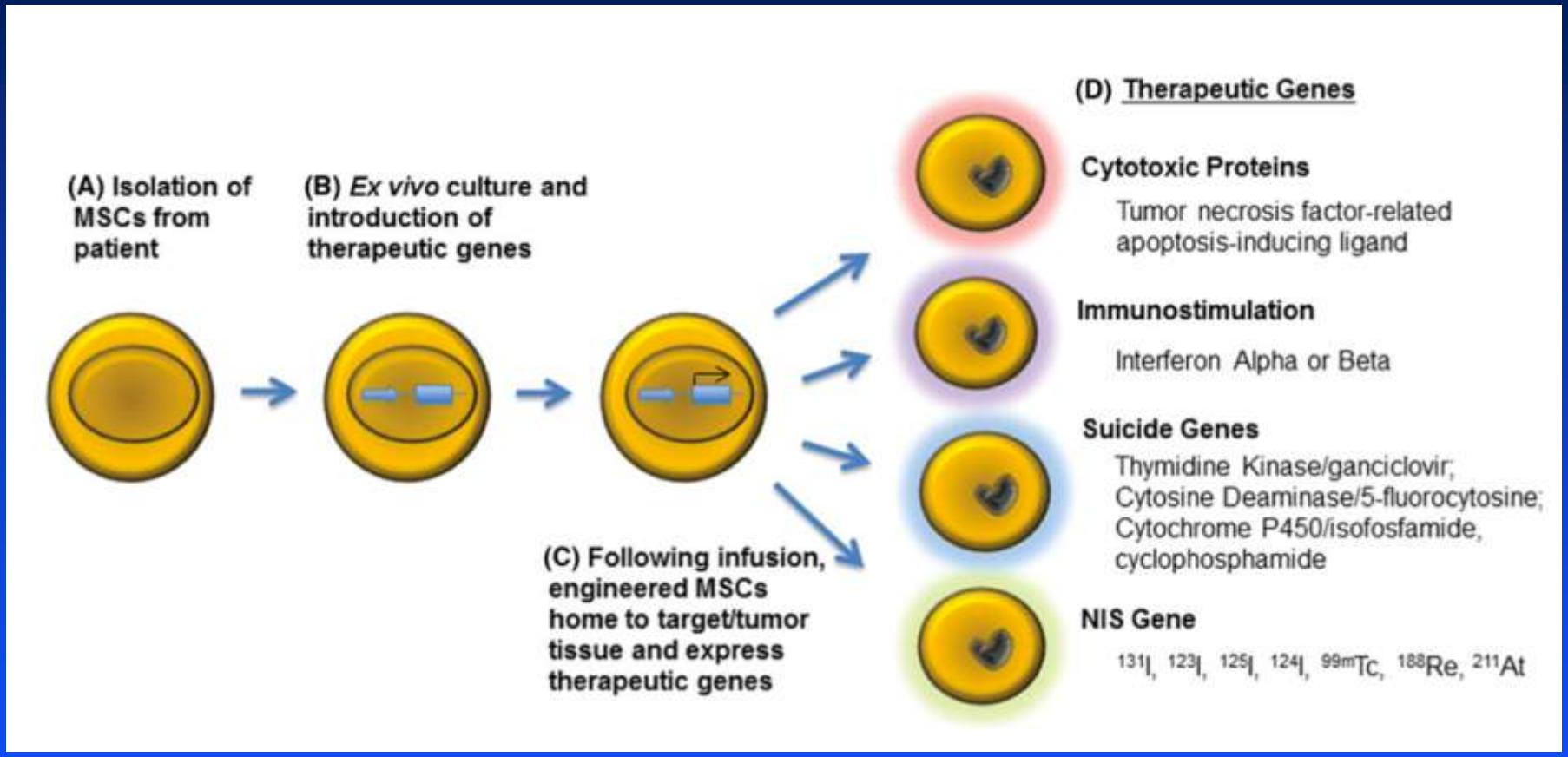
- MSCs will accumulate at tumor sites
- Interferon-β expression in melanoma
- Also IFN-α, IFN-β, IL-2 and IL-12
- Ad-IL12-MSC showed antimetastatic effect
- Express pro-apoptotic genes
- Tumour necrosis factor-related apoptosis inducing ligand (TRAIL)
- Prodrug converting enzymes
- Tyrosine kinase (HSV-tk)
- Cytosine deaminase (CD)

MSCs and Gene Therapy Strategies

- MSCs can protect viruses from neutralizing antibodies
- Carriers for delivery of oncolytic viruses
- Attenuated measles virus
- Conditionally replicating oncolytic adenoviruses (CRAds)
- MSCs can be transduced with the sodium iodide symporter (NIS) gene, infused, and the patient then treated with ^{131}I radionuclide

Ong et al. J Hepatol 2013; Knoop K et al. Hum Gene Ther 2013

MSCs and Gene Therapy Strategies



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Other Progenitor Stem Cells for Therapy

- Liver stem cells can migrate to HCCs both in vitro and in vivo
- Multipotent adult progenitor cell/endothelial progenitor cell (MAPC/EPC) subpopulation isolated from bone marrow can engraft in highly vascular tumors
- These MAPC/EPCs may be used for endothelium-specific gene therapy

The Way Forward

- There is much work needed to standardize methods of isolation, characterization and expansion of MSCs
- There is substantial concern about possible oncogenic effects of MSCs, including risk of insertional genotoxicity and secondary cancers
- Techniques are being developed to enhance targeting of MSCs to tumors
- MSCs remain an attractive potential tool for targeting tumors both for treatment and for prevention of recurrence

Thank You

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