Tamara LahhalaL amaraT

Doktor Jekyll in gospod Hyde

Dvojna narava matičnih celic v raku
Dual nature of stem cells in cancer
Adam and Eve - first human stem cell on Earth was the Source of Good and Evil
Hypokrates: … 430 BC

Karcinom – rak (καρκίνομα) … cancer –

- Hippocrates described the vital force, called “nature” (φυσις – physis) as the internal driving force of vital functions, which is the best remedy that should be reinforced by doctors –

- Hints at the existence of stem cells, and a suggestion to be used to cure an organism by cell therapy?!
Normal stem cells in humans take care of (limited) tissue regeneration

Embryonal
Fertilised egg

Adult/tissue stem cells:
Mesenchymal
Haematopoietic
Mesenchymal Stem Cells are Part of Tumour Microenvironment Potential Use in Cell Therapy of Cancer?

In the regenerative medicine: preferential use of MSC (vs ESC, IPS), as multipotent MSC are not tumorigenic in vitro and in vivo in normal tissue.
Good Dr. Jekyll…

- Cancer therapy?
- Immune diseases
- Bone and cartilage
- Neurodegenerative diseases
- Heart and vascular damage
- Skin, burns, plastic surgery
- Radiation damage

Used alone - and as vectors for drugs – more efficient!
Robert Louse Stevenson 1886 novel

Strange case of Dr. Jekyll and Mr. Hyde

Dr. Henry Jekyll and his alternative personality, Mr. Edward Hyde is one fictional character who occasionally feels he is battling between the good and evil within himself, thus leading to the struggle between his dual personalities of Dr. Jekyll and Mr. Hyde.

Can mesenchymal stem cells turn to another, different state and acquire genetic set up of cancer?
As each cell, also the stem cell can be damaged, or the well-differentiated damaged cell can become stem cell... 

cancer stem cell.

What is a stem cell?

A single cell that can

replicate itself, or...

differentiate into many cell types.

The 2012 Nobel Prize in Physiology or Medicine was awarded jointly to Sir John B. Gurdon and Shinya Yamanaka "for the discovery that mature cells can be reprogrammed to become pluripotent"
A Paradigm Shift: Asymmetric Division and Hierarchical Order of Cancer Evolution

Stochastic Hierarchical models of tumor development

Metastases are the Cause of Patients’ Death: Migratory Cancer Stem Cells – CSC:

“Seed and Soil Hypothesis”
Sir Stephen Paget - 1885

sCSC sit quietly in theirs niches

mCSC cells – genetic change
Start moving!

Form metastasis is they find their new tissue niches

Visvader and Lindema, 2010
GLIOBLASTOMA

Histology:
- angiogenesis
- necrosis
- no metastasis
- growth & invasion

Oncology:
- ↑ Prevalence 3-5/100 000 inhabitants
- ↓ Survival (avg cca 16 months)
- (?!?) Surgery, Radiation, Chemotherapy, etc.
- New (cell) therapies?
What do we investigate?

GBM STEM CELLS – EVIL MR. HYDE
Origin and evolution of GBM stem cells: GSC

Tumour initiated cells acquire stem cell characteristics

Tumour associated niches-hypoxic

De-differentiation
Proliferation
Mutation

Verbovšek et al., Trends in Stem Cell Proliferation, 2013

Bjerkvig et al., Stem Cell & Cancer Biology, 2009
Niches: Problem with Glioblastoma Stem Cells Resistance

Primary GBM  Minimal residual  Recurrent GBM avg16 months

Perivascular niches

Searching for GSC Niches in GBM Sections

Glioblastoma stem cells

CD133 /prominin1
GBM SC marker

GSC niche markers:
• **CD133** and nestin as GSC markers
• **SMA** as smooth muscle cell marker
• **SDF-1α** as chemotactic cytokine involved in GSC maintenance and retention in niche

Breznik, in review JHC
Cathepsins K and X, together with B, are localized in peri-arteriolar GSC Niches

Cathepsin B: the substrates Z-Ala-Arg-Arg-4MβNA acetate
Cathepsin K activity: Z-Gly-Pro-Arg-4MβNA acetate on cryo-sections were used
Cysteine Cathepsins are family of 11 proteolytic enzymes normally located to lysosomes and upregulated in many cancers.

FUNCTION?
Katepsin K and GSC niches

CD9 – NEW ! marker for GBM Stem Cells

Transmembrane protein CD9 is glioblastoma biomarker, relevant for maintenance of glioblastoma stem cells

Tetraspanin CD9 stabilizes gp130 by preventing its ubiquitin-dependent lysosomal degradation to promote STAT3 activation in glioma stem cells
Dr. Jekyll created a serum, or potion, in an attempt to mask this hidden evil within his personality. However, in doing so, Jekyll transforms into Mr. Hyde, a hideous, evil creature mysterious and violent. As time goes by, Hyde grows in power...become stronger than Jekyll...
CONFRONTATION!

Cancer stem cell & Endothelial cell In periarterial niche

Tumor heterogeneity refers to stromal & various types of tumour cells present in the tumour mass

Mesenchymal Stem Cells MSC
DOCTOR JEKYLL becomes MR HYDE cell type?

Transition to malignancy:
  Cell contact independent
  Cell contact dependent:
  * Indirect
  * Direct
Trans-differentiation

Immunomodulation

paracrine effects—cytokines

PROMOTE
Proliferation
EMT: Motility, Invasion
Metastasis
Apoptosis Inhibition
Angiogenesis
Immunosuppression
Increase tumor-initiating cells
CCL5, TGF-β, IL-6, IL-10, VEGF,
MMP, SDF-1, Neuregulin

INHIBIT
Oncostatin M
Tumour growth
Immunoprotection,
Anti-angiogenic effect

Positive and negative effects of MSC on tumour progression observed are depending on experimental set up, MSC origin and cancer type
Perversion of stem cell potential of pericytes.. MSC become CSC!

Brain mesenchymal stem cells: The other stem cells of the brain?

Florence Appaix, Marie-France Nissou, Boudewijn van der Sanden, Matthieu Dreyfus, François Berger, Jean-Paul Issartel, Didier Wion
Cell Fusion

U87/MSC (30% of cells)

Cell Cannibalism – entosis

U373/MSC (3%) of cells

Mona Oliveira
Bone marrow-derived MSCs

U87dsRED + U373eGFP

GBM INVASION

EXPRESSION of candidate proteases (qPCR, flow cytometry and Western blot)
- Cathepsins B, L, K and Calpains P1,2
- uPA/uPAR, MMP-2, -9 and -14

VALIDATION by selective inhibitors
I. INVASION

MSCs inhibited invasion of U87 cells and increased invasion of U373 cells out of mixed spheroids in vitro
MSCs impaired U87 cells’ but enhanced U373 cells’ invasion upon co-injection into the zebrafish embryos
**Summary** Protease cascades in MSC-GBM direct contact

**MSC-impaired U87** cell migration and invasion correlated with **decreased** levels of **MMP-14** and **uPAR**

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<thead>
<tr>
<th>U87</th>
<th>MONO-CULTURE</th>
<th>DIRECT CO-CULTURE</th>
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<tbody>
<tr>
<td>INVASION</td>
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<td>MMP-14</td>
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**MSC-enhanced U373** cell migration and invasion correlated with **increased** levels of **cathepsin B**, **MMP-9**, **MMP-14**, **uPA/uPAR**, but not **cathepsins L and K**

<table>
<thead>
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CONCLUSIONS & COMMENTS

MESENCHYMAL STEM CELLS are potential MR HYDE in cancer due to:

- Direct transdifferentiating to Cancer Stem Cells
- Affecting certain types of tumour cells to be more invasive and aggressive:
  - Secreting cytokines - their poisonous serum!
  - Attaching to them and changing their genes directly
  - Forming novel fused hybrids cells - novel monstrous Mr Hyde!

MSC CAN ONLY BE USED AS TROJAN HORSES to deliver poisonous vectors to tumour cells (suicidal enzymes–apoptosis signals) and strictly subtypes- selective treatment!

PROTEASES
Potential use of multiple-general PAN- protease inhibitors, as adjuvant treatment to MSC in selective (mesenchymal) glioblastoma subtypes could be recommended.
Hvala za pozornost!
Thank you for your attention