Human uterus leiomyoma tissue for invasion studies

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My dream: To create a model for analyzing the invasion process: the interaction between human carcinoma cells and tumor microenvironment matrix using human tumor tissue in vitro

The **Classic Collagen 3D-Organotypic Model**

"rat-mouse-man"

(since 1980’s; Fusenig NE et al.)

- Human cancer cells
- Rat tail type I collagen + Mouse EHS-tumor Matrigel + Human Fibroblasts
- Nylon membrane
- Medium (+ e.g. inhibitors)
- Steel grid

Problems with classic 3D "rat-mouse-man" model

"Comparing murine with human tissue structures suggest, that even the structural and physical parameters diverge" 

(Wolf et al. Seminars in Cell and Dev Biol, 2009)

Human tumor microenvironment model = **Myoma model:**

Uterine Bening Smooth Muscle Neoplasm

- Myoma is common: incidence 20 – 50 %
- Most likely to be diagnosed with leiomyomas at the age of 40 – 50 yr
- Easy to obtain as a left-over material after the tumor operation

Cutting myoma slices
8 x 4 mm punch biopsy slices of an average size myoma will give ~125 discs

**Composition of Myoma tissue (IHC)**

**Matrix molecules:**
- Hyaluronic acid
- Collagen types I, III, IV
- Laminins

**Cells:**
- VIM= fibroblasts
- SMA= smooth muscle cells
- CD 45 and CD 68 = inflammatory cells
- FVIII = endothelial cells

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**Growth pattern of OTSCC cells in myoma vs. 3D organotypic collagen model?**

**Over 100 myomas are currently tested:**
HSC-3 invasion varies in different myoma discs

**Most of the cells in myoma discs are nonvital** (apoptotic) after storage in liquid nitrogen

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8 cm

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Tongue SCC, patient sample

**HSC-3** in myoma disc

**HSC-3** in fbl + collagen gel


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**TUNEL** (**green**) & DAPI (**blue**), Roche

**ApopTag**, Chemicon

**Growth pattern:**
Tongue carcinoma cells invade mostly in budding pattern (less than 5 cells in a group); whereas mucoepidermoid carcinoma cell line invades in collective clusters into myoma disc.

HSC-3
Highly invasive tongue SCC

MUC-1
Mucoepidermoid carcinoma cells

**HSC-3 cells invade up to 7x deeper in myoma than in the 3D collagen + fibroblasts**

![Graph showing invasion depth comparison between HSC-3 cells in myoma and collagen.](image)


... but they proliferate less (Ki-67 index) in myoma than in collagen

![Graph showing Ki-67 index comparison.](image)


Some cancer cells are positive for both mesenchymal (VIM) and epithelial (AE1/AE3) markers in myoma. Sign for epithelial-mesenchymal-transition (EMT)

VIM

AE1/AE3

AE1/AE3 + VIM


In myoma discs, not in collagen, ICTP-RIA measures the invasion depth of HSC-3 cells

**Type I collagen fragments**

![Graph showing inhibition of invasion and type I collagen degradation with MMP inhibitor GM6001.](image)

Nurmenniemi et al. 2009

“Rinsing media” from intact myoma tissue

12 % SDS-PAGE: media samples collected from days 2, 5, 7

Are soluble factors relieved during rinsing period involved in invasion?
Yes - Rinsing of myoma tissue affects SCC cell invasion!

"Anti-invasive" arresten transfected HSC-3 cells
Aikio et al. Plos One 2012

3D Collagen gel+fbl No invasion
Myoma discs were rinsed for 14 days in DMEM w/o serum before the experiment with the SCC cells

Hypoxic conditions induced HSC-3 invasion in rinsed myoma, but

Hypoxic conditions induced HSC-3 invasion in rinsed myoma, but

Invasion depth
Rinsed Myoma Intact Myoma

... intact myoma provides the best hypoxic TME for HSCC-3 invasion. Why?
Teppo et al., Exp Cell Res 2011

Invasion inducing and hypoxia factors are present in intact myoma tissue!

MMP-11
Myoma tissue extract w/o HSC-3 cells: Western blot
Intact Rinsed
MMP-11 has pro-invasive & anti-apoptotic properties.

LOX-1
Myoma tissue extract w/o HSC-3 cells: western blot
Intact Rinsed
LOX is secreted by hypoxic tumours; facilitates invasion and metastases formation.
(Effer et al. Nature 2007)

Teppo et al., Exp Cell Res 2013

Some examples of the published results using myoma invasion assayss

The myoma model has been used in more than 20 publications. So far it has not been criticized by reviewers 😊

Lymph node metastatic, more aggressive cell line (SCC-9 ZsG LN-1) of the primary tongue carcinoma (SCC-9ZsG) cells invaded significantly deeper into myoma

SCC-9 ZsG SCC-9 ZsG LN-1

Agostini M et al. Mol Cancer Ther. 2014

Human bone marrow derived mesenchymal stem cells (hBMMSC) do not invade in myoma
CpG induced invasion!

Non-CpG CpG

Toll-like receptor 9 activation with CpG-oligonucleotide enhances the invasion of hMSCs compared to activation with non-CpG

Nurmenniemi et al. Exp Cell Res. 2010

2/7/2016
An increase in MMP-13 synthesis is detected in the CpG-activated MSCs; addition of MMP-13 antibody significantly diminished the CpG induced invasion.

MMP-13 (arrow) in invading MSCs into myoma

- MMP-13 participates in MSCs invasion

Trypsin-2 transfected HSC-3 cells invaded deeper in myoma than controls - MM-14 specific inhibitor (peptide G) reduced the invasion

Peptide G is a selective MMP-14 peptide-inhibitor (GACFSIAHECGA)
- It do not affect the activities of MMP-1, 2, 3, 7, 8, 9, 10, 11, 12, 13, 15, 17 or 20
- The peptide effectively inhibited the migration and (Transwell) invasion of cancer cell lines and reduced the growth of tongue carcinoma xenografts in mice. [Suojanen et al. 2009]

MMP-14 induces HSC-3 invasion

Oral dysplastic cells (DOK) did not invade in myoma model, not even after co-culturing with gingival fibroblasts (GF) or with mesenchymal stem cells (MSC)

Conforming the expression of molecules – Myoma model in a laser capture method

The expression of cat K was confirmed by RT-PCR of the RNA isolated from lazer captured cells from either frozen sections or from paraffin embedded samples

"Hitching a ride" mechanism

"In SCC, carcinoma associated fibroblasts (CAFs) are always the leading cell of the invading cohort with the SCC cells closely following behind."

In the myoma model:
- bone marrow derived mesenchymal stem cells (MSC) and SCCs are mixed:
- Some of the MSCs are leading the invading SCCs colonies

Co-cultures of macrophage subtypes and HSC-3 in myoma

THP-1 leukemic cells were induced to macrophages (M) with:
- PMA+ LPS + IFN-γ → M1-M (pro-inflammatory)
- PMA+ IL-4 + IL-13 → M2-M (pro-tumorigenic)

Depth of HSC-3 invasion measured from myoma

HSC-3 + M1-M → reduced
HSC-3 + M2-M → induced HSC-3 invasion compared to HSC-3 cells alone
What to do with discarded discs and the left-over myoma tissues?

What if we prepare Myogel similar to Matrigel®?

**Basement membrane extracts (BME) prepared from EHS mouse sarcoma tissue**
(Patent for Matrigel ended yr. 2006)

**Composition:**
- Laminin
- Collagen
- Heparin sulphate proteoglycan,
- Entactin/nidogen
- Growth factors (TGF-β, EGF)

*Adhesion experiments*
*Invasion (3D)*
*Drug screening*
*Angiogenesis (tube formation)*
*Xenografts*
*Directed differentiation*

**Matrigel®, ECMatrix™, Cultrex®, BME®, Geltrex®**

**Other human ECM extracts prepared similarly**
- **Skeletal muscle myogel**
  adipogenic and support the ex vivo amplification of corneal epithelial cells

- **Amnion tissue extract** (Amgel, HumBiogel)

- **MaxGel™ Human ECM (Sigma), AlphaMAX3D (Neuromics)**
  co-culture of fibroblasts and keratinocytes
  -> matrix for BME extract

**Human myoma -> Myogel**
**Mouse EHS-tumor -> Matrigel®**

*A novel human leiomyoma tissue derived matrix for cell culture studies*
Proteomic of Myogel vs. Matrigel

Myogel: out of 765 identified proteins 34% were the same as in Matrigel® and 66% were different.

Myogel was lacking some growth factors and MMPs present in Matrigel®.

Myogel had a number of ECM and connective tissue forming proteins lacking in Matrigel®.

The pH:
Myogel is neutral & more stable than Matrigel®

Myogel with SCC cells | Matrigel® with SCC cells | Myogel without cells
---|---|---
0 h | 7.0 - 7.5 | 8.0 - 8.5 | 7.0 - 7.5
17 h | 7.0 - 7.5 | 8.0 | 7.0 - 7.5
48 h | 6.5 - 7.0 | 6.0 - 6.5 | 7.0 - 7.5

Thin coating: adhesion
Carcinoma cells adhere most to Matrigel

Coating the bottles: none, BSA, Matrigel, Myogel + HSC-3 cells

Thin coating: gene expression
Carcinoma cells expressed genes differentially on top of Myogel vs plastic

Uncoated | Myogel coated
---|---
Affymetrix GeneChip Human Genome U133 Plus 2.0 Arrays
RNA harvested after 24 h HSC-3 cells collected
Totally 1.4% (751) genes were differentially expressed (FC 1.5 or more)
Mostly pathways were related to intracellular organelle cytoskeleton organization biogenesis
Significant changes related to actin polymerization and reorganization

Thin coating: horizontal migration
The HSC-3 migration is faster on Myogel than on Matrigel®

Thick coating: Transwell invasion

Human cancer cells (50,000/well) Transwell insert
Myogel
Nylon filter membrane Medium +chemoattractant
Invading cells
Incubation for 12 to 48 hours
**Matrigel vs. Myogel vs. No gel**

HSC-3 cells invade more efficiently through Myogel than Matrigel

<table>
<thead>
<tr>
<th>Concentration of Myogel and Matrigel was 2.4 mg/ml</th>
<th>Salo et al. BMC cancer 2015</th>
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**Thick coating with Myogel-LMA:**

- Low Melting Agarose (LMA) is added to Myogel for keeping it in gelatinous form
- Cells did not invade through LMA w/o myogel

**Thin and thick coating:**

Scratch wound healing assay with IncuCyte

More cell lines are invading through Myogel than Matrigel (unpublished)

**Hanging drop spheroids:**

Carcinoma (HSC-3) cells form larger colonies within Myogel-LMA than in plain conventional low melting soft agarose (LMA)

**Hanging-drop assay:**

Myogel-collagen, Matrigel-collagen or collagen
HSC-3 cells move faster in Myogel-collagen matrix than in Matrigel®-collagen matrix in the 3D hanging-drop cultures.

Possible applications for "Myoma Kits" - A part of cancer drug testing?

Drug sensitivity and resistance testing (DSRT) of Pa02c

IC50 of 132 tested compounds against Pa02c

Viability of the drug-test cells:

Angiogenesis:

Myogel efficiently induces endothelial cell tube formation: more, smaller & longer lasting (up to 72h) tubes compared to Matrigel-GFR or ECMatrix

1. No coating
2. Matrigel (0.62 mg/ml)
3. Myogel (0.62 mg/ml)

Three days incubation

Viability measuring by CellTiter-Glo®

Luminescence detection using the PHERAstar®

Data analysis by Dotmatics (EC50, IC50)

132 compounds
"drugs for leukemia"
5 concentrations each

IC50: no coating = Matrigel = 57%
IC50: no coating > Matrigel = 27%
Drug more effective in Matrigel
IC50: no coating < Matrigel = 16%
Drug more effective on plastic (unpublished)
My dream: Myogel - a commercial product replacing at least partially the use of Matrigel

- Adhesion experiments
- Invasion (3D)
- Drug screening
- Angiogenesis
  - Xenografts?
  - Directed differentiation?
  - What else?

- Patent application is filed
- TEKES TUTLI project (8/2015-1/2017) to further evaluate the properties of myogel and possibilities for producing Myogel as a commercial product

But: Collaborative work is still needed before we can evaluate if Myogel would be a useful multipurpose product

Researchers and collaborators in myoma projects

**Oulu group**
Pia Nylund, PhD, docent
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Katja Pukala, MSc

**Oulu collaborators**
FIMM collaborator
Weinberg group

**Brazil collaborators**
Coletta, Graner and Leme groups

Current financial support

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