Effects of non-cellular tumor microenvironment matrix on oral cancer prognosis and in vitro experiments

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Non-cellular matrix in primary oral cancers

Structural proteins
Enzymes
Active fragments
Growth Factors
Extracellular vesicles
Cytokines

Matrix molecules are produced and modified by subclones of cancer cells & cells in tumor microenvironment (TME)

Stiffness of TME:
Type I collagen synthesis (PINP-ab) in OTSCC prognosis

Higher PINP expression in invasive vs superficial areas associated with worse prognosis of OTSCC patients; HR 1.524, 95% CI 1.272-1.815 p=0.000
Salo S. et al 2013

High PINP expression at the invasive front CAFs associated with a poor prognosis of OSCC patients; HR 3.341, 95% CI [1.54-5.91] p=0.002
Bagiokakis et al 2016

Ligands in TME:
TN-C and FN in OTSCC prognosis

Tenacin-C (TN-C) & Fibronectin (FN)
Both are present in most solid tumors
Both are related to cell adhesion & migration

5 year survival rate in early stage OTSCC:
TN-C: 88% if TN-C was not in TME
42% if TN-C was abundant in TME
FN: 100% if FN was not in TME
24% if FN was abundant in TME

Conclusion:
Expression of FN and TN-C in the TME, not in SCC cells, differentiate patients into low- and high-risk groups
Sundquist et al. 2017

The prognostic value of tumor to stroma ratio (TSR) and budding & depth of invasion (BD) in oral tongue cancer

Wu et al. TSR Meta- analysis. Oncotarget 2016

Stroma-rich group:
In multivariate analyses
- Worse disease-free survival: HR 1.91 (95%CI 1.17-2.79, P= 0.008)
- Higher cancer-related mortality
  HR 1.71 (95%CI 1.02-2.86, P= 0.03)

Combination of the highest-risk parameter scores for TSR and BD:
- Disease recurrence
  HR 3.42 (95%CI 1.71-6.82 P< 0.004)
- Cancer-related mortality:
  HR 11.6 (95%CI 3.83-35.31 P< 0.001)

Conclusion:
Should we add the analyses of TSR and BD to pathology reports of OSCC?
Almangush et al. 2018
**Cytokines in TME:**

**IL-17F in OTSCC prognosis**

- IL-17F belongs to IL-17 cytokine family; greatest homology with IL-17A
- Extracellular, not intracellular - amount of IL-17F in IF front mast cells
- Disease specific survival

In multivariate analyses:
- HR 4.18 (95% CI 1.01-17.26, p=0.04) for early stage
- HR 3.51 (95% CI 1.48-8.34, P=0.004) for all stages

**Conclusion:**

Extracellular mast cell-derived IL-17F in the TME has anti-tumorigenic effects in OTSCC

Almahmoudi et al. 2018

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** Structural changes in negative LNs architecture of the patients with early stage OSCC**

- A thickened capsule & many reactive cortical follicles
- > prognosis is better

- A thin capsule and a few reactive cortical follicles
- > prognosis is worse

Vered et al. 2014

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**3D TME invasion models to analyze the interaction between carcinoma cells and TME**

1) Organotypic “rat-mouse-man” model, since 1980’s (Fusenig et al.)

- Human cancer cells
- Rat tail type I collagen
- Mouse tumor (Matrigel)
- Human fibroblasts
- Nylon membrane
- Medium
- Steel grid

**Human has 78 less proteases than mouse**

Overall & Cohen-Otm 2002

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2) Human uterus myoma disc model (Nurmenniemi et al. 2009)

- Human cancer cells in myoma disc
- HSC-3 tongue carcinoma cells in myoma disc
- HSC-3 + fibroblasts in collagen disc
- HSC-3 cells invade 7 times deeper in myoma than in the collagen + fibroblasts discs

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**TME in cancer patients’ lymph nodes:**

structure & composition are different from the primary tumor

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**In vitro solid 3D matrix models for oral cancer invasion studies**

Cloudberry
**HSC-3 cells do not invade within pig tongue, human heart, or nose polyp tissues. Why?**

**Myoma is a mesenchymal tumor containing natural TME which is essential for carcinoma cell invasion**

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**Every myoma is “individual”**

- Invasion of the index cell line (HSC-3) varies in different myoma discs

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**SEM and TEM of HSC-3 cells in intact myoma**

- Protrusion of the membrane (arrow) surrounding the “nesting” SCC cell in the upper part of the myoma; breaks in BM

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**Rinsing of myoma tissue affects SCC cell invasion**

- “Anti-invasive” arr-HSC-3 cells (transfected with arresten)

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**Myoma discs simple to prepare and use**

- Human Tumor Tissue Based 3D in vitro Invasion Assays: Åström et al. Methods Mol Biol, 2018

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**Myoma discs composition**

- Collagen types I, III, IV, laminin
- Hyaluronic acid

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**Myoma discs simple to prepare and use**

- Around 120 discs by punch biopsy from an average size myoma
**Intact Myoma is hypoxic and contains several invasion inducing factors**

- CA-9 (hypoxia factor carbonic anhydrase IX)
  - CA-9 in HNSCC is associated with reduced survival
- MMP-11
  - MMP-11 has pro-invasive & anti-apoptotic properties
- LOX
  - LOX is secreted by hypoxic tumors; facilitates invasion

**Solid myoma discs:**

- used in studies related to various cancer cell lines or co-culture experiments

  - The gene transfection - invasion
    - Åström et al. 2017, 2018
  - miRNA/miRNA/protein identifications in invasive vs non-invasive cells
    - Lazzer capture
    - Korva et al. 2017
  - Myoma +/- cells irradiation +/- drugs
    - Vökip et al. manuscript
  - Co-cultures: cancer cells +/-
    - # M1 or M2 macrophages
    - Pirsk et al. 2015
    - # Activated mononuclear cells
    - Alsmadi et al. 2017

**Effects of myoma, collagen+fbl, or no matrix on carcinoma cells growth pattern in mice**

A. Myoma +/- HSC-3
B. Collagen gel + fibroblasts +/- HSC-3
   - precultured for 10 days
   - Discs were transplanted onto the dorsal muscle fascia
C. Subcutaneous (inj). HSC-3
   - After 6 weeks 6 alb-c nude nu/nu mice in group A and B were killed
   - Mice in group C were sacrificed when the tumor volume reached 1,000 mm³
   - Implants and xenografts were collected in 4% formalin and embedded in paraffin

**Same cancer cells in nude mice:**

- different growth patterns - depends on the TME

- Stromal reaction
- EMT
- Invasion
- Dysplasia
- Invasion
- Encapsulation
- Necrosis

**For faster in vitro analyses gelatinous mesh matrices are needed**

**Matrigel®**

- the "golden standard" for cancer in vitro studies

**Interview 2013:**

“Are you surprised that Matrigel was so successful?”

Hynda Kleinman (NH, USA):

“I’m shocked that it’s this useful. I’m also shocked that no one has invented anything better. It’s still made the exact same way we made it 25 years ago. There must be thousands of tumors to make it. Nobody’s done that.”

**Matrigel = basement membrane extract**

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

A mouse natural tumor: the composition varies from lot to lot!
We prepare Myogel similar to Matrigel

Proteomic analyses

765 identified proteins:
- 34% were the same in both
- 66% were different
- Myogel: more small proteins than Matrigel
- Myogel mesh is looser than Matrigel

Potential use of Myogel for cancer patient’s tissue/blood samples in pre-treatment tests

We compared their properties in cancer invasion assays

Spheroid wells are imaged and measured daily for:
- Spheroid size (minus original size)
- Invasion distance (spikes)
- For drugs and irradiation analyses

Why still 95% of anti-cancer compounds which have promising effects in preclinical studies fail in Phase III clinical trials?

Could we do something better in HTS?

We tested 12 HNSCC cell lines with HTS method against 19 anti-cancer drugs

Heat map of the drugs tested on cancer cell lines on top/within Myogel, plastic, Matrigel

EGFR inhibitors

12 HNSCC cell lines

No effect -> highly effective

19 drugs against:
- Epidermal growth factor receptor (EGFR)
- Mitogen-activated protein kinase (MEK)
- Phosphoinositide 3-kinases (PI3K)
- Mechanistic target of rapamycin (mTOR)
3.7.2018

### Phase III clinical trials for Erbitux
- Response rate was 13 %

<table>
<thead>
<tr>
<th>Response rate for Erbitux (%)</th>
<th>Control</th>
<th>Matrigel 2D</th>
<th>Matrigel 3D</th>
<th>Myogel 2D</th>
<th>Myogel 3D</th>
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<td>Mean response rate of all the 12 HNSCC cell lines tested: Control &amp; Matrigel 2D 68%, Myogel 2D 15%</td>
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**Monotherapy response objective patients rate clinical trials for HNSCC.**

Tuomainen et al. unpublished

See poster #206


- *Future pre-clinical drug screening & response rate testing?*

**Sporadically or organotypic models**

**Tests with human primary and metastases TME mimicking matrices**

**Towards Personalized Medicine**

**Conclusion:**

The structure and composition of the non-cellular ECM plays a crucial role in cancer growth both in vivo & in vitro

**Should new active compounds be tested in cancer cell lines on top of or embedded in human tumor matrix (Myogel) wells?**

**Future pre-clinical drug screening & response rate testing?**

**Rat type collagen**

**Human Myogel**

**Mouse Matrigel**

**Bovine serum albumin**

**Human fibrin**

**Human fibronectin**

**Researchers and collaborators behind these projects**

**Oulu group: past and present members**

- Maija Risteli PhD, Johanna Konsta PhD, Mauricio Dourado PhD
- Elias Sundquist PhD, Ilka Alahauté PhD, EiranbHooper APS
- DDS,Sirsa Salo PhD, Sini Nummenenimi PhD, Susanna Teppo MSc
- Meeri Syttinen PhD, Pia Nyberg PhD, Emma Pirilä PhD
- Arja Keränen PhD, et al

**Oulu collaborators**

- Petri Lehenkari, prof. group
- Ilkka Miinalainen, PhD

**Helsinki group**

- Ahmed Al-Samadi PhD, Alhadi Almangush PhD
- Katja Tuomainen MSc, Rabia Almuhaidib PhD
- DDS
- Helsinki Biobank collaborators
- Paivi Saavalainen, doc. group
- Antti Kallaste, prof. group
- FIMM collaborators
- Krisier Wenerberg, prof. group

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- Ricardo Coletta, prof. group
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