Patient derived models for Breast Cancer

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Presentation Overview

- Introduction to the limitations of current models of cancer
- In house data supporting the 3D spheroid culture as a model for human breast cancer
- New orthopic *in vivo* models for breast cancer
- Conclusion and outlook
The R&D Process
long, complex, costly…

- Cost of **inventing** for a new medicine up to **$1 billion**. Process takes up to 12 years

- Considering money spent on drug failures, the average cost to **bring a drug to market** is closer to $4 billion (to bring a drug to the market cost in average from $3.7 –Amgen– and $12 billion –Astrazeneca–)

- About **1 in 5 of drugs** (antibodies) tested in humans make it to the clinic (1 in 20 for other chemical compounds)

Low efficacy / cost ratio
Misinterpretation of Preclinical Data

- Compounds which are biologically active but do not block progressive disease were pushed in clinical studies! TGI 1
- Only compounds which induce regression (in relevant models) should be pushed in clinical development! TGI 2
- Standard human tumor xenograft models do not reflect expression of target in normal tissues
A Disease: Model vs Reality

Major differences between standard mouse tumors and cancers in patients

Topics

Large number of cells
- Homogeneous
- Single site
- Rapid growth
- Restricted
- Weeks
- Before metastasis

Tumor initiation
- Cellular composition
- Primary neoplasms
- Growth rate
- Genetic background
- Therapy duration
- Initiation of therapy

Clonal form a single cells
- Heterogeneous
- Multiple sites
- Slow growth
- Diverse
- Months
- After metastasis

Requirements for a tumor model:
Similarity to human biology to ensure predictability for clinical trials
“Everything should be made as simple as possible, but not simpler.”

Albert Einstein
Breast cancer is not a single disease, but is instead a collection of diseases that have:
- distinct histopathological features,
- genetic and genomic variability, and
- diverse prognostic outcomes.

*These are the most common profiles for each subtype. However, not all tumors within each subtype will have all these features.

<table>
<thead>
<tr>
<th>Subtype</th>
<th>These tumors tend to be*</th>
<th>Prevalence (approximate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal A</td>
<td>ER+ and/or PR+, HER2-, low Ki67</td>
<td>40%</td>
</tr>
<tr>
<td>Luminal B</td>
<td>ER+ and/or PR+, HER2+ (or HER2- with high Ki67)</td>
<td>20%</td>
</tr>
<tr>
<td>Triple negative/basal-like</td>
<td>ER-, PR-, HER2-, cytokeratin 5/6 + and/or HER1+</td>
<td>15-20%</td>
</tr>
<tr>
<td>HER2 type</td>
<td>ER-, PR-, HER2+</td>
<td>10-15%</td>
</tr>
</tbody>
</table>

→ no individual model would be expected to completely recapitulate this complex disease.
Advantages and Limitations of current Breast Cancer Models

In vitro

2D models

- Easy to handle
- Good for studying molecular mechanisms
- Mostly cell line derived
- Absence of tumor microenvironment
- Homogeneous

3D models

- Good for studying molecular mechanisms
- Mostly cell line derived
- Heterogeneity is lost after passages
- Absence of tumor microenvironment

In vivo

Xenografts

- Easy to handle
- Fast growing tumors
- Histopathological properties different to patients
- Limited stromal infiltration
- Consist largely of proliferating epithelial cells

GEMMs

- Intact immune system
- Spontaneous tumor development
- Limited availability
- Labour intensive and slow
The Predect approach
an European effort

Generation, characterization, and reciprocal comparison
The Predect approach

3D Spheroids

Roche contribution

Intraductal

Generation, characterization, and reciprocal comparison
Goals for the project at Roche
Generate patient derived *in vitro* and *in vivo* models of breast cancer

**Human primary tumors**

**Advanced In vitro model**

*3D spheroids from primary breast cancer samples*

- Generate patient derived 3D cultures
- Keep expression profile of markers expressed within the primary tumor
- Use as a source for *in vivo* xenografts

**Orthotopic In vivo model**

*Intraductal vs fat pad tumorgraft models*

- Generate patient derived BC models
- Obtain similar BC phenotype as the primary tumor
Simplified process outline

Human Breast tumor
(average sample) → 3D Spheroids → Mice:
Intraductal vs fat pad transplantations
**TAFs**

Collagenase / trypsinization

60 min 37°C

Mince Tumor Cells (-TAFs)

**Sample for initial characterization**

**BC Sample**

**Mince**

**Collagenase / trypsinization**

60 min 37°C

**Tumor Cells (-TAFs)**

3D cultures/Co-cultures

**Semi-solid matrix**

Suspension (low attachment plates)

- Medium 1
- Medium 2
- Medium 3
- …

Intraductal vs fat pad transplantation

**Description of the Process**
Culture of primary 3D spheroids in floating suspension

Medium 3 supports culture: 6/7 samples
Medium 3 allows 3D spheroids to get propagated longest: up to P7 (~8 months)
The ability of forming 3D spheroids in medium 3 may be stem cells related?

→ Medium 3 is the most advantageous
→ Limitation: 3D spheroids in suspension can be propagated but not amplified
→ Technology needs to be optimized!
Culture of primary BC cells using Semi-solid matrix

Both matrices allow spheroids:
- propagation for more than 8 months in culture, P9+ (ongoing)
- growth with both 2D and 3D characteristics

→ Limitation: 3D spheroids on semi-solid matrix can be propagated but not amplified
3D culture
3D spheroids amplification

- 3D spheroids can be amplified ≥ 3 fold
- Further optimization ongoing

Matrix 1

Matrix 2

Optimized culture
Propagation

Amplification

Amplification
ER expression is maintained in amplified 3D spheroids, however it is less intense than in the primary tumor.
Simplified description of the process

Human Breast tumor
(Average sample) → 3D Spheroids → Mice:
Intraductal vs fat pad transplantations
Advantages of intraductal transplantation compared to intra fat pad injection:

- Only cell line based models for intraductal exist (e.g. DCIS.com)
- Tumors developed by intraductal transplantations show phenotypical properties (histology) closer to real breast cancers, as cells are injected into the cellular, biochemical and biophysical environment where BC arise (DCIS.com)
Conclusions

- **Patient derived 3D spheroids can be kept in culture for several months**
  - In both: suspension and semi-solid matrix
  - In semi-solid matrix they are maintained for 8+ months (ongoing)

- **3D spheroids can be amplified**
  - Currently limited (~3 fold), optimization ongoing

- **Maintenance of ER expression**
  - Lost in classical culture systems
  - Retained in our culture system
Optimize amplification → for model use *in vitro* and *in vivo*

- Extend IHC characterization, include: HER2, PR and ki67
- Assess the functionality of ER
- Analyze cellular composition, stem cells markers
- Study more samples, different passages
- Reciprocal comparison of model and tumor characteristics
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