The Concept of Cancer and Metastasis stem cells

EUSJA Meeting
EMBL/DKFZ
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What distinguishes CANCER from other diseases that are curable such as Tuberkulosis, Cholera, Hepatitis A or Aids?
Cancer is a **GENETIC** Disease
Every tumor and every patient is individual:

- sequencing of 500 tumors of 50 cancer types will be sequenced
- Genome: $2 \times 10^9$ BP : 200- volume Encyclopedia with 2000 pages
  -> Cancer: one page is missing, two sentences mixed up and 30 typos...
- starting 2015-20: DKFZ/NCT: Sequencing of any incoming patient
- development of targeted therapies to all major pathways
- significantly improved drug efficacy with less side effects
Colon Cancer

Evolution: Yachida et al Nature 2010
Classical Cancer Therapies

- Surgery
  - Chemotherapy
  - Radiation
  - Targeted Therapies
(3) Targeted Therapy

- Targeted therapies influence pathways that are deregulated in the cancer cells, but not in normal cells.

-> Signalling pathways which are altered due to the mutations (in oncogenes and tumor suppressors) are inhibited.
   → High cancer cell specificity and little side effects!

-During the last 10 years such substances have been introduced into clinical practice and hundreds of clinical trials are currently under way to test newer and better ones.
(3) Targeted Therapy

- Tyrosine-kinase-Inhibitors: Gleevec® (Imatinib, Dasatinib, Nilotinib...). Very successful use in CML (chronic myeloid leukemia) and Gastro-intestinal-stromal tumors (GIST)
Lasker~DeBakey Clinical Medical Research Award 2009

Brian Druker   Nicholas Lydon   Charles Sawyers

For the development of molecularly-targeted treatments for chronic myeloid leukemia, converting a fatal cancer into a manageable chronic condition. (More >)
(3) Targeted Therapy

Monoclonal Antibodies:

- Erbitux® (Cetuximab): anti-EGFR
- In combination with chemotherapy for colon and head and neck cancer

- Trastuzumab (Herceptin®, Roche): anti-HER2/neu
- Breast Cancer, but only the ones who have HER-2 overexpressed (about 25%)
Monoclonal antibodies

(3) Targeted Therapy

Immune cells targeting cancerous cells bound by Herceptin

With Herceptin

Herceptin is the only approved HER2 therapy designed to bind to HER2+ tumor cells and flag them for destruction by the immune system

Without Herceptin

Dimerized HER2 receptors signal tumor cells to proliferate

Herceptin blocks downstream HER2 signaling to inhibit proliferation of cells
Promising, but still in clinical studies:

**Lung Cancer:**
- Crizotinib: Tyrosine Kinase Inhibitor
  - ca. 5% of lung cancer patients express an EML4-ALK Fusionsprotein (Non-Smoker)
  - large trials ongoing

**Melanoma:**
- B-Raf Inhibitor (PLX4032)
  - only efficient for 2-18 months!
  - mechanism of resistance is understood
(3) Targeted Therapy

- High specificity, thus less side effects

- only rarely as monotherapy (Gleevec), mostly in combination with Chemo

- Each tumor and each patient is different:
  - Patients must be tested whether the target is expressed
  - therapy is only useful in some patients
  - Personalized Medicine (→ costs (!))
Krebs: Eine Krankheit der Gene

Neueste umfassende Studien von Tumor-Genomen (Dickdarmkrebs, Brustkrebs, Glioblastome) zeigen:

- unerwartet hohe Anzahl an Mutationen (> 15-20 kausale Mutationen)
- jeder Tumor ist verschieden

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Adult stem cells are essential for the life-long maintenance and repair of regenerative tissues.

- Every two months-
- Every week « 200g » -
- > 2 Billion cells/day! -
Regenerative tissues are built by three basic cell types:

- **Stem cells**
  - Self-renewal (life long)
  - Pluripotent
  - Very rare
  - Long-lived
  - Infrequent divisions
  - Require interaction with the stem cell niche

- **Transit Amplifying cells**
  - Rapidly dividing
  - Intermediate lifespan
  - Multipotent

- **Mature cells**
  - Non-dividing
  - Short lifespan
  - Terminally differentiated
  - Essential for the physiology of the tissue
Novel drugs that eliminate cancer stem cells

Tumor loses its ability to constantly produce new cells and eventually degenerates

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Conventional chemotherapy: Kills tumor cells but may spare cancer stem cells

Tumor initially shrinks by 99% but relapses

Novel drugs that eliminate cancer stem cells

Tumor loses its ability to constantly produce new cells and eventually degenerates

---

(Modified from Reya et al., 2001)
Cancer: The Dandelion Problem

→ Excellent success rate – at least initially......
....not only Cancer Stem Cells,
also normal Stem Cells
survive Chemotherapies
(well otherwise...!)
Chemotherapy eliminates proliferating cells, whether they are tumor or normal cells- but SC seem resistant!

- **Stem Cells**
  - Self-renewal
  - Expansion

- **Chemotherapy**
  - Proliferating Progenitors
  - Terminal differentiation

- **Terminally Differentiated Cells**
  - Bone marrow
  - Skin
The story of Gleevec and Chronic Myelogenous Leukemia (CML)

Gleevec targets (BCR-Abl carrying) leukemic progenitors but **not** the CML leukemic stem cell, since patients rapidly relapse after stopping Gleevec!
The story of Imatinib and Chronic Myelogenous Leukemia (CML)

Gleevec targets (BCR-Abl carrying) leukemic progenitors but not the CML leukemic stem cell, since patients rapidly relapse after stopping Gleevec!
Minimal residual disease are caused by dormant metastasis stem cells and may be re-activated even more than 10 years after the initial treatment.
Adult blood stem cells (HSCs) are known to be “SLOW CYCLING”, but are some of them QUIESCENT or even long term DORMANT? and what is their behavior during HOMEOSTASIS and INJURY?
The adult murine hematopoietic system

Modified from Passegué E. et al., 2003, Yang et al., 2005, Kiel et al., 2005
Stem Cells: less than one in 100’000 cells !!!
How do we tag cells with fluorescent markers?

- **Fluorescent tag**
- **Cell receptor**
- **Cell surface**

Diagram:
- Stem cell
- Fluorescent tag attached to a cell receptor on the cell surface.
FACS ≠ FAX 😊

Flourescence Activated-Cell Sorting

Up to > 100’000 cells/minute

FLUORESCENT ACTIVATED CELL SORTING (FACS)

Laser beam passes through one cell

A cell generates a negative charge if it fluoresces and a positive charge if it does not.

GRAPHIC DISPLAY OF FACS

Fluorescence intensity

Stammzelle
Purity of an HSC containing population can be determined by single cell transplantations followed by assaying for multi-lineage long-term reconstitution.
Dormant HSCs show the highest repopulation activity

**LSK**

- **dHSC**
  - DORMANT (5 Div/LT)
  - LRC-HSC
  - high SR activity
    - > serial
  - CD34 mRNA\(^{lo}\)
  - Replication off

- **aHSC**
  - ACTIVE (1 Div/Month)
  - Non LRC-HSC
  - low SR activity
    - > only 1°
  - CD34 mRNA\(^{hi}\)
  - Replication on

- **MPP1**
- **MPP2**
- **MPP3**
- **MPP4**

Similar results:
(Hanno Hock et al (2008), Nature Biotechnology)
(Kateri Moore et al (2009), unpublished)
Dormant and self-renewing HSCs

What is their role in the body? Emergency, in response to injury? --> treat mice with 5-FU!
Dormant HSCs are recruited into the cell cycle in response to injury signals.

dHSCs (LRC\(^{GFP204}\)CD\(^{34\text{neg}}\)150\(^{+}\)48\(^{\text{neg}}\)LSK)

![Flow cytometry plots showing cell cycle distribution before and after treatment with 5-FU. The plots indicate a change in the percentage of cells in G0, G1, S/G2/M phases before and after treatment.]
Bone marrow harbors a reservoir of deeply dormant but highly potent HSCs which can be reversibly activated in response to injury cues.

15% subset of the HSC (CD34-150+48-KLS) population

Dormant
Niche
Hypoxic
Low metabolism

(Trumpp, Wilson and Essers, NRI 2010)
Acute stimulation with IFN$_{\alpha}$ activates dormant HSCs

(M. Essers et al. (2009), Nature advanced online pub. 11. Feb. 09)
Interferon-α (IFNα)

• Member of the type I interferon family
• Inhibits virus replication, enhanced IFN production during viral infection
• Immunomodulatory activity
• Principally anti-proliferative (but many clinical effects of IFNα remain “mysterious”)

IFNα in the clinic:
• Hematological malignancies
  chronic myeloid leukemia (treatment of choice before Imatinib),
  cutaneous T cell lymphoma, hairy-cell leukemia, multiple myeloma
• Viral syndromes
  hepatitis C, hepatitis B, severe acute respiratory syndrome

Most cancers acquire resistance to long-term, high dose IFNα therapy; nevertheless some patients achieve a long-term cure
  - the mechanism for this phenomenon however remains unknown!
Acute Stimulation with IFNα activates dormant HSCs and sensitizes them to Chemotherapy

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Acute IFNα

Dormant Stem Cells

Active Stem Cells

Progenitors

Self-renewing HSCs (tissue maintenance)

expansion

CT-sensitive

CT-sensitive

CT-sensitive

IFNα-priming experiment

+ PBS + 5-FU weekly survival
IFNα-priming experiment

+ PBS  + 5-FU  survival

+ IFNα  + 5-FU  Death due to HSC depletion?
HSCs can be eliminated by IFNα priming followed by 5-FU treatment

- Lethality accompanies pan-cytopenia and total HSC loss
- Same 5FU experiments with IFNR−/− mice: all mice survive!
IFNα-priming activates all functional HSCs

+ IFNα + 5-FU

Death due to HSC depletion

• Strong correlation between IFNα induced HSC cycling and 5-FU sensitivity

• If dormancy is the main reason why cancer stem cells (CSCs) are resistant to anti-proliferative therapy, IFNα priming might be a novel way to eliminate such CSCs!
IFNα may also activate dormant CML stem cells making them sensitive to imatinib.
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Dormant CML-SCs are resistant to imatinib

Activated CML-SCs may be sensitive to imatinib

More differentiated CML cells are effectively eliminated by imatinib.

“minimal residual disease”
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More differentiated CML cells are effectively eliminated by imatinib.

Case report: six patients who have been switched from IFNα to imatinib treatment did not relapse after stopping imatinib! (Rousselot et al., 2007)

Erst aufwecken.. …dann abtöten....
The Putative Metastasis Initiating Cell (MIC)

Are MICs a subset of "Circulating Tumor Cells" (CTCs)? How complex are CTCs?

The presence of CTCs correlates with bad prognosis in breast cancer.

The number of EPCAM+ CTCs before treatment is an independent predictor of PFS and OS in patients with metastatic breast cancer (Christovanilli et al., NEJM 2007).
The phenotype and biology of CTCs are (almost) a “black box”

• Are CTCs heterogeneous?

• Can subpopulations be identified and isolated by FACS?

• Are CTCs actively dividing?

• Do (some…) CTCs behave as metastasis initiating cells (MICs) and can strategies been developed to detect and eliminate them?
Study with 600 metastasized breast cancer patients: CTC Quantification using CellSearch System
Number of CTCs in metastatic breast cancer patients (n=180)

- 0 to 4: 70.27%
- 5 and more: 29.73%
- 5 to 50: 20.95%
- 51 to 100: 4.05%
- 101 to 500: 3.38%
- 501 to 1000000: 1.35%

Total: 8.78%
FACS analysis of CTCs isolated from a metastatic breast cancer patient

Type: Luminal A
Receptor status: ER+PR+Her2-
Metastasis: bone, heart
CTCs: 4000 / 7.5 ml blood
Patient CTCs are heterogeneous containing a small $CD44^{hi}CD24^{lo}$ population

$CD44^{pos}CD24^{lo}$: Putative breast cancer stem cell phenotype  
(Al-Hajj and M. Clarke, PNAS 2003)

Three luminal A/B patients with bone metastasis
GATED ON LIVE CD45neg CTCs

PATIENT 1

PATIENT 2

PATIENT 3
CSC-CTCs can be in a quiescent G₀ phase
Circulating tumor cells (CTCs) and Metastasis?

Breast cancers metastasize preferentially to:
bone, liver and lung

Metastasis Stem Cell
- Express targetable receptors and a “do not eat me signal”
- can be quiescent/dormant
PRESENT
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Ines Brückmann
Larissa Carnevalli
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Major fields of Interest:

- **Interferons in LSCs** (Marieke Essers)
- **Fanconi and blood reprogramming** (Mick Milsom)
- **Cancer and Metastasis Stem Cells** (Andreas Trumpp and Martin Sprick)
- **Metastasis SC Proteom and Biomarkers** (Christoph Rösli)

"Heidelberg Institute for Stem Cell Technology and Experimental Medicine"

www.hi-stem.de