Targeting the Notch Pathway: Killing Cancer Stem Cells

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Outline

• Background
  • Cancer Stem Cells
  • The Notch Pathway

• Targeting agents
  • Drugs
  • Antibodies

• Challenges

• Future Directions

• Conclusions
Cancer Stem Cells

• Definition: Cells within a tumor that possess the capacity for self-renewal, differentiation, and tumorigenesis when implanted into an animal host

• First identified in 1994 in human acute myeloid leukemia (AML)
  • Cells were transplanted into severe combined-immune deficient (SCID) mice and tumors formed

• Found for first time in human solid tumors (breast, brain) in 2003
Cancer Stem Cells

- Possess characteristics of both stem cells and cancer cells
  - Undergo asymmetric cell division generating daughter cells where one retains stem-like properties and one progresses through cell division and differentiation
- Make up small a portion of the overall tumor
  - Up to 20% of cells in some solid tumors have been identified as possible CSCs
- Still some debate about origin of cancer, are CSCs real?
- More evidence mounting in favor of CSCs: including the heterogeneity that is characteristic of many tumor types
Origins of Cancer- Hypotheses

Cancer cells

Tumor development

No tumor

Proliferation and differentiation of the CSC

No tumor

CSC

The Cancer Stem Cell (CSC) or hierarchical model

Stochastic model

Other types of cancer cells

Cancer Stem Cells
Cancer Stem Cells- Identification

- CSCs have been identified in various tumor types: AML, brain, breast, colon, liver, prostate, ovarian, pancreatic, bone, kidney, skin, and head and neck cancers
- Identified by markers that vary depending on the tumor type
  - Cell surface proteins
  - Cellular activity
- Not exact: not unique to CSCs (also in normal stem cells of the tissue)
- No single marker is known to easily identify CSCs

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute Myeloid Leukemia (AML)</td>
<td>CD34⁺ CD38⁻</td>
</tr>
<tr>
<td>Brain Cancer</td>
<td>CD133⁺ CD15⁺</td>
</tr>
<tr>
<td>Breast Cancer</td>
<td>CD44⁺ CD24⁻/low CD133⁺</td>
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<tr>
<td></td>
<td>ALDH-1⁺</td>
</tr>
<tr>
<td>Liver Cancer</td>
<td>CD133⁺ CD90⁺ CD45⁻</td>
</tr>
<tr>
<td>Prostate Cancer</td>
<td>CD44⁺ α2β1 integrin&lt;sup&gt;high&lt;/sup&gt; CD133⁺</td>
</tr>
<tr>
<td>Pancreatic Cancer</td>
<td>CD44⁺ CD24⁺ ESA⁺</td>
</tr>
</tbody>
</table>
Cancer Stem Cells- Identification

1. Patient samples have cell subpopulations sorted by flow cytometry based on markers present or not present
2. Cells baring the desired markers are transplanted into immunocompromised mice
3. Allow tumors to grow (if CSCs present)

OR

1. Patient samples are diluted down to low concentrations
2. Take aliquots and implant into immunocompromised mice
3. Allow tumors to grow (if CSCs present)
Cancer Stem Cells- Identification

1. Patient sample cells are sorted for desired markers
2. Plated at low density on a solid substrate
3. Allow colonies to grow (if progenitor or CSCs present)

OR

1. Patient sample cells are sorted for desired markers
2. Suspended in a semi-liquid medium at low density
3. Allow spheres to grow (if CSCs present)
Cancer Stem Cells- The Problem

- Chemo- and radio-resistance
  - Chemo: Post-treatment HER2\(^+\) breast cancer biopsy samples were enriched in CD44\(^+\)CD24\(^{-}\)/low cells
    - Before: 4.7% vs after: 13.6%
    - Ability to form mammospheres before: 13.3% vs after: 53.5%
  - Radio: lower levels of ROS were detected in CSC enriched human breast cancer samples after radiation relative to non-CSC populations
    - Higher levels of expression of several ROS scavengers (superoxide dismutase 2, SOD2, methionine sulfoxide reductase A, MSRA) were found in CSC enriched samples

- Metastasis
  - CD44\(^+\) breast CSCs from primary tumors and secondary lung metastases resulted in metastasis when transplanted into immunocompromised mice

- Recurrence
Cancer Stem Cells - The Problem

A. Conventional Therapies

Conventional therapies target the bulk of the tumor, but resistant cancer stem cells (CSCs) remain viable, leading to tumor relapse.

B. CSC-Targeted Therapies

CSC-targeted therapies eliminate CSCs, leading to the degeneration of the tumor.

Cancer Stem Cell (CSC) Differentiated Tumor Cells

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Bioessays 2009, 31, 1038-1049

Date: 1/2/2016
Cancer Stem Cells- Targeting

• Due to slow growth/replication rate, many standard chemotherapeutics won’t work
• CSCs are heavily reliant on pathways that control self-renewal, embryonic development, and differentiation
  • Wnt
  • Hedgehog (HH)
  • Notch
  • Transforming Growth Factor β (TGF-β)
• Theoretically, inhibiting these pathways should target CSCs, selectively killing them
• Killing these “seed” cells should make tumors vulnerable to standard treatments
The Notch Pathway

• Originally discovered in *Drosophila melanogaster*
• Highly conserved embryonic developmental pathway
• Cell-to-cell contact is required for Notch signaling between cells
  • Ligands are presented on one cell and the receptor on the other
• 4 Notch receptors (Notch 1-4)
  • Single pass transmembrane cell surface receptors
  • Active receptors are heterodimers that originate from a single precursor which is cleaved then reconnected non-covalently
• 5 Notch ligands (Delta-like ligand [DLL] 1,3,4, Jagged [JAG] 1,2)
• Gene targets include HES and HEY transcriptional repressors, and NF-κB
The Notch Pathway
Breast Cancer

• Notch signaling activity in breast cancer is common, up to 50% of cells have Notch activation
• Notch 1 activation resulted in accelerated tumor growth *in vivo*
• High JAG 1 and Notch 1 expression levels are correlated with poor patient survival
• Reduction in Notch 1 and 4 activity lead to reduction in amount of CD44^+^/CD24^low^ cells
  • Also reduced tumorigenicity after transplantation into immunocompromised mice
The Notch Pathway

- In most tissues, maintains an undifferentiated state
- In several tumor types, the expression level of Notch pathway components positively correlated with tumor grade
- Aids in CSC survival
- Plays a role in Epithelial-Mesenchymal Transition (EMT)
- Contributes to chemoresistance

Carcinogenesis 2013, 34, 1420-1430
Targeting Agents- Merck

• **MRK-003**
  - The preclinical/nonhuman compound used to guide the development of another Merck GSI, MK-0752
  - Originally tested against Alzheimer’s disease for its ability to block Aβ40 accumulation
    - Subnanomolar activity against γ-secretase (0.24 nM)
    - *in vivo* efficacy against Aβ40 accumulation
  - Orally bioavailable

![Chemical Structure of MRK003](attachment:image.png)

Targeting Agents - MRK-003

• Kondratyev et al. treated primary tumor cells with either DMSO or MRK-003 for 4 days

• Injected cells from the spheres that formed into mice subcutaneously and allowed tumors to grow

• All animals were sacrificed when a tumor that was 10% of the weight of any occurred
Targeting Agents- MRK-003

- Tanaka et al. treated cells derived from GBM patient sample neurospheres with MRK-003
- MRK-003 induced apoptosis more vs DMSO control
- Lower left quad (red circle) = viable cells
- Upper right quad (purple circle) = apoptotic cells
• Tanaka et al. treated patient derived samples with DMSO or MRK-003 for 7 days
• MRK-003 resulted in significantly decreased tumorosphere formation relative to DMSO control
• Upper panel is from MRK “relatively sensitive” tumor
• Lower panel is from MRK “relatively resistant” tumor
Targeting Agents- Merck

• **MK-0752**

  • Originally tested against Alzheimer’s disease for its ability to block Aβ40 accumulation
    • $IC_{50} = 5 \text{ nM}$
    • *in vivo* efficacy against Aβ40 accumulation
  • Orally bioavailable

• Has been in 9 clinical trials mostly Phase I, but also Phase I/II
  • Either as a single agent or in combination
Targeting Agents- MK-0752

- Schott et al. transplanted patient derived metastatic breast cancer cells into immunodeficient mice
- Treated with either vehicle or MK-0752 (100 mg/kg po) for 3 days

A. Cells were harvested and allowed to grow mammospheres
   - Significantly reduced mammosphere formation in both primary and secondary assays relative to vehicle control

B. Cells were assessed for CSC markers (CD44+/CD24-)

\[ A \]

\[ B \]

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Targeting Agents- MK-0752

- Schott et al. transplanted patient derived metastatic breast cancer cells into immunocompromised mice
- Treated with either vehicle, MK-0752 (100 mg/kg), docetaxel (10 mg/kg), or both
  - Clinically relevant dosing schedule shown
- Significant difference between MK-0752 treatment and vehicle
- Significant difference between docetaxel alone and combination treatments
Targeting Agents- Clinical Trials
MK-0752

- 5 Completed clinical trials
  - For patients with advanced cancers
  - In combination with Gemcitabine, Ridaforolimus, or Docetaxel
  - Well tolerated in pediatric patients

- 3 Terminated clinical trials
  - Mainly due to toxicity and lack of efficacy

- 1 Active clinical trial for the treatment of early stage breast cancer in combination with Tamoxifen Or Letrozole

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Clin. Cancer Res. 2013, 19, 1512-1524
ClinicalTrials.gov
Targeting Agents– Clinical Trials
Roche

• RO4929097

  • Investigated in both Alzheimer’s disease and cancer
    • γ-Secretase IC$_{50}$ = 4 nM
    • More selective inhibition for Notch cleavage over Aβ40
  • Selective (>100 fold selectivity over 75 tested proteins)
  • Orally bioavailable

• Has been in 35 clinical trials, both Phase I and Phase II
  • Either as a single agent or in combination

• No longer in development by Roche
• Huynh et al. treated aggressive melanoma cancers with either DMSO or RO4929097 (10µM)

A. mRNA levels were determined for the downstream target gene of HES1 in treated and untreated cells
   • RO4929097 inhibits the Notch pathway *in vitro*

B. Treatment with RO4929097 resulted in significantly reduced cellular proliferation
Targeting Agents - RO4929097

- Huynh et al. transplanted WM3248 melanoma cells into immunocompromised mice
  
  A. The expression levels of melanoma CSC markers were determined
  
  B. Primary tumor cells were collected from vehicle and RO4929097 treated mice and transplanted into new mice
  - Secondary tumors were untreated with additional compound
Targeting Agents- Clinical Trials
RO4929097

- Lee et al., in a Phase II trial in metastatic melanomas, found that:
  - RO4929097 was well tolerated
  - 1 Partial response lasting 7 months
  - 8 patients had stable disease lasting at least through week 12
    - 1 continued for 31 months.
  - The 6-month progression-free survival rate was 9%
  - The 1-year overall survival rate was 50%
  - Lack of inhibition of Notch target genes
Targeting Agents- Clinical Trials RO4929097

- Diaz-Padilla et al., in platinum resistant ovarian cancers, found:
  - Also found RO4929097 to be well tolerated
  - 33% of patients reached stable disease (median duration = 3.1 months)
  - Median progression free survival (PFS) of study was 1.3 months (1.2-2.5)
  - Median PFS was higher when Notch intracellular domain detection was high (3.3 months vs 1.3 months for low NICD)
Targeting Agents- Clinical Trials
RO4929097

- 15 Completed clinical trials
  - Typically for patients with advanced, metastatic, recurrent, or difficult to treat tumors
  - In various combinations including with other chemotherapeutic agents, radiotherapy, or surgery
- 13 Terminated clinical trials
  - Mainly due to too few subjects and discontinuation of RO4020097
- 4 Withdrawn clinical trials before recruitment occurred
- 3 Active clinical trials
  - For glioblastoma, breast cancer, and melanoma
Targeting Agents - Clinical Trials Pfizer

- PF-03084014

  - Originally tested against Alzheimer’s disease for its ability to block Aβ40 accumulation
    - Good cellular activity against γ-secretase (1.2 nM)
    - *in vivo* efficacy against Aβ40 accumulation
  - 13.3 nM activity against Notch cleavage
  - Selective for γ-secretase
  - Orally bioavailable
Targeting Agents - PF-03084014

- Wei *et al*. transplanted leukemia cells into immunocompromised mice
  
  A. PF-03084014 treatment (50 mg/kg) resulted in significantly decreased levels of free NICD
  
  B. PF-03084014 treatment resulted in reduced tumor volume
    - 92% reduction with 150 mg/kg dosing
• Yabuuchi et al. transplanted pancreatic cancer cells into immunocompromised mice
  • In their 7 patient samples the percent CSCs varied greatly: 0.64 - 16.6%
• Treated with gemcitabine (25 mg/kg) or PF-03084014 (150 mg/kg) or both
  • Gemcitabine alone increased amount of CD44+/CD24+ cells
  • PF-03084014 alone decreased amount by 3.7%
  • Combination decreased amount by 3%
Targeting Agents– Clinical Trials PF-03084014

• 3Withdrawn clinical trials
• 4Terminated clinical trials
  • All terminated June 24, 2015 due to “change in strategy of development”
  • “There were no safety/efficacy concerns”
• 1Active clinical trial
  • Phase II
  • Single agent, for desmoid tumors

Celeste Alverez @ Wipf Group

ClinicalTrials.gov
Clin. Cancer Res. 2015, 21, 60-67
Targeting Agents– Clinical Trials
Bristol-Myers Squibb

• BMS-906024
  - Selective
  - IC$_{50}$ = 2-3 nM
  - Cellular IC$_{50}$ (TALL-1) = 4 nM
  - *in vivo* ED$_{50}$ < 0.5 mg/kg in TALL-1

• BMS-986115
  - Orally bioavailable
  - Both in Phase 1 trials: Currently recruiting

ACS Med. Chem. Lett., 2015, 6, 523-527
ClinicalTrials.gov
LY900009
IC₅₀ = 0.27 nM
Solubility = 0.06 mg/mL (@ pH 2, 6, and 7.4)
Metabolically labile
- Especially the aliphatic tail
- CYP3A4 primarily responsible for metabolism

Need to improve metabolic stability, remove active metabolites, maintain potency

1 completed clinical trial - data not disclosed
Targeting Agents - Clinical Trials
Eli Lilly

• 2nd Generation compound

• IC\textsubscript{50} = 0.31 nM
• Solubility = 0.20 @ pH 2.0, 0.22 @ pH 6.0, 0.02 mg/mL @ pH 7.4
• Metabolism significantly reduced
  • Major metabolism still by CYP3A4
  • Major metabolite N-dealkylation - more active than parent
• **LY3039478**

• IC<sub>50</sub> = 0.41 nM

• Solubility = 0.89 @ pH 2.0, 0.88 @ pH 6.0, 0.88 mg/mL @ pH 7.4

• Metabolism significantly reduced

• No active metabolism identified in metabolism screen

• Good to moderate clearance (species dependent)

• Bioavailability: mouse, rat, dog; 65-67%

• In Phase I clinical trial
  - No preclinical data disclosed
The Notch Pathway - Targets

Nature Reviews | Clinical Oncology
Targeting Agents- Clinical Trials

Anti-DLL4

- It has been shown that inhibition of DLL4 results in non-productive angiogenesis
  - Leading to hypoxia at tumor and decreased tumor growth
- DLL4 inhibition has also been shown to be anti-tumorigenic via mechanisms other than angiogenesis
  - *in vitro* blockage of DLL4 via mAb resulted in reduction of Notch signaling

![Graph showing Luciferase Reporter Activity](image)
Targeting Agents- Clinical Trials
Anti-DLL4

- OMP-21M18
- Monoclonal antibody (mAb)
  - Selective for DLL4 over other Notch ligands
- Treatment of a colon tumor xenograph with OMP-21M18, irinotecan, or both resulted in reduced tumor volume compared to the control
- After stopping dosing tumors were allowed to continue growing (B)
  - Those treated with no OMP-21M18 continued to grow, OMP treated cells did not
Targeting Agents- Clinical Trials

Anti-DLL4

- **OMP-21M18**
  - 1 Completed phase I clinical trial
  - 2 Active phase I clinical trials currently running
    - Single agent or in combination
    - For Non-small cell lung cancer and pancreatic cancers
  - 1 Currently recruiting clinical trial in combination with paclitaxel for the treatment of Pt resistant ovarian cancer

- **MEDI0639**
  - mAb, currently recruiting for phase I clinical trial for advanced solid tumors

- **REGN421**
  - mAb, completed phase I clinical trial for advanced solid tumors
The Notch Pathway - Targets

Neighbouring signal-sending cell

Neighbouring signal-sending cell

Ligand degradation or recycling

Extracellular space

Delta-like (DLL1, DLL3, DLL4) or Jagged (JAG1, JAG2) ligands

Notch1-4

Activated receptor

Signal-receiving cell

Cytoplasm

NICD

γ-secretase complex

Notch target genes

HES family

MYC

CDK inhibitor (CDKN1A)

Cyclin D3 (CCND3)

HER2

NRARP

Nucleus

Nature Reviews | Clinical Oncology
There are currently 2 anti-Notch antibodies in clinical trial

**OMP-52M51**
- Binds Notch 1
- Selective over other Notch receptors
- 2 Phase I clinical trials currently recruiting

**OMP-59R5**
- Binds Notch 2/3
- Tested preclinically in a variety of tumor types with good efficacy
- Tested in combination with chemotherapeutics with good efficacy
- 1 Active phase I clinical trial
- 2 Phase Ib/II clinical trials currently recruiting
Targeting Agents

• Notch pathway inhibitors alone are at best moderately effective
• Because they will in theory only kill CSCs, it would be ideal to combine these compounds/antibodies with another chemotherapeutic agent which results in non-CSC cell death
• Combinations of GSI+chemotherapeutic as well as antibody+chemotherapeutic have been/are being tested to varying degrees of success
• GSI treatment also can lead to toxicity
  • It was found co-treatment with a glucocorticoid could reverse this toxicity and does not hinder efficacy
Potential Alternate Targets

![Diagram of Notch signaling pathway](nature-reviews-clinical-oncology-2015-12-445-464)
Current Challenges - Toxicity/Efficacy

- Toxicity associated with Notch inhibition
  - One of the major reasons many GSIs do not make it past the preclinical/early clinical stages is due to dose limiting toxicity (DLT)
  - Most common DLT for GSIs is diarrhea and other gastrointestinal symptoms
    - Notch inhibition can cause differentiation of intestinal goblet cells and reduced absorption
    - Glucocorticoids can reverse this toxicity
- Efficacy at times looks poor in the clinic due to Notch targeting treatments being used in tumors with no CSCs, investigators need to screen
Current Challenges - Identification

• There is no universal CSC marker
  • Markers for each tumor type must be determined individually
  • Not all tumors of a given tissue express the same markers in different individuals
• There is no simple method of determining CSC content within a patient tumor
  • It can be unreliable to assess CSC presence via blood testing - not all markers are present in the blood
  • Identifying cell surface markers requires biopsies and then secondary typically indirect means to determine if CSCs are present
  • Or the assumption that the cell surface markers truly indicate CSCs
  • Assays to determine CSC presence tend to be based on secondary tumorigenicity
    • does not necessarily indicate CSCs, might be progenitor cells
• Difficult to assess any potential treatment without
What More Can Be Done?

• Alternative targets need to be explored:
• GSIs may not be the most efficient way to target CSCs
• Targeting protein-protein interactions should be considered
• Jagged 1 has also been implicated in CSC activation—consider it as a target
• Possibility for small molecule inhibition of Notch-ligand binding
Conclusion

- CSCs have been identified in various tumor types by phenotypic and molecular means
  - CSCs contribute to chemo- and radio-resistance, metastasis, and recurrence
  - Targeting embryonic pathways essential to the survival of CSCs should be a viable means of killing them
- The Notch pathway has been implicated in the survival of CSCs
- By targeting γ-secretase there has been some success preclinically and clinically in targeting and killing CSCs, especially as part of combination therapies
- Targeting Notch and Notch ligands has also been explored via mAb and several clinical trials are underway to determine safety and dosing
- Determining a straightforward mean of identifying CSCs may be the key to targeting Notch in CSCs and moving drugs beyond the clinic
Acknowledgements

• Dr. Wipf
• Wipf group members past and present
Chemoresistance of CSCs

Effect of chemotherapy on the mean percentage of cells that express high levels of CD44 and low levels of CD24 (CD44+/CD24-/low) among HER2-negative patients. Percentage of tumorigenic cells increased at week 3 ($P < .001$) and remained high at surgery (week 12) ($P < .001$).

Effect of chemotherapy on mean mammosphere (MS) forming efficiency before, during, and after treatment. All patients, $P < .001$. 

Radioresistance of CSCs

Human breast cancer from which CD44$^+$CD24$^{-/low}$Lin$^-$ cells (cancer stem cell enriched population, red) and “Not CD44$^+$CD24$^{-/low}$” Lin$^-$ non-tumorigenic cells, green, were isolated using flow cytometry. Intracellular ROS concentrations were subsequently measured by DCF-DA staining for the two populations.

- ROS levels were significantly lower in CSC enriched sample
Representative H&E stains of sections from the breast tumor (BT), dissected lungs, and lymph nodes (LN) from a NOD/SCID mouse transplanted with CD44+ human triple negative breast cancer tumor cells (Magnification: 200×)

*Proc. Natl. Acad. Sci. USA 2010, 107, 18115-18120*
The problem with CSCs and Notch

Primary Tumor → Primary Tumor with Metastatic Potential → Cancer Stem Cells with Drug Resistance → Distant Metastasis

EMT → Conventional treatment → MET

Growth factors, TGF-β, Notch 1, and Wnt

Drug Sensitivity → Drug Resistant

Adapted from: Cancers 2011, 3, 716-729
Targeting Agents – Clinical Trials
Merck
Targeting Agents– Combinations

A

<table>
<thead>
<tr>
<th>Veh</th>
<th>PF-03084014 (14 d)</th>
<th>Dex (14 d)</th>
<th>PF-03084014 (14 d)</th>
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</thead>
<tbody>
<tr>
<td>H&amp;E</td>
<td>Alcian blue</td>
<td>Ki67</td>
<td>H&amp;E</td>
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Mol. Cancer Ther. 2010, 9, 1618-1628