Oncogene Inactivation (Addiction) and CD4+ T-cells

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SITC Workshop 2012
Modeling Oncogene Addiction Inside and Out

Oncogene Addiction is both Tumor Intrinsic and Host-Dependent

Mechanisms of Oncogene Addiction and the Immune System

Restoration of Tumor Intrinsic Fail-Safe Mechanisms:
  Shut Down of Self-Renewal Programs: Role of Cellular Senescence

Restoration of Host Fail-Safe Mechanisms:
  Shut Off of Angiogenesis

Importance of Immune System and Autocrine/Chemokine Signaling

Combining Targeted Therapeutics + Immune Therapy
Figure 2 | Strategies for the therapeutic activation and enhancement of senescence. Schematic view of the approaches that are readily available for the implementation of pro-senescence therapy in cancer treatment. Inhibitors are shown in red boxes and target proteins in blue ovals. a) Enhancement of p53 activity through either inhibition of the interaction between MDM2 and wild-type p53 (for example, a nutlin) or restoration of mutant p53 activity (for example, PRIMA-1^MT or ellipticine). b) Modulation of cell cycle machinery, for example through either inhibition of S phase kinase-associated protein 2 (SKP2) or cyclin-dependent kinase 2 (CDK2). Both approaches result in increased p27 (also known as KIP1) activity and a consequent senescence response. c) Induction of senescence in tumours that are addicted to MYC through the inhibition of the MYC oncogene in combination with an immunomodulatory approach. d) Induction of PTEN loss-induced cellular senescence (PICS) through inhibition of PTEN and consequent mTOR-mediated activation of p53. e) Induction of replicative senescence through inhibition of telomerase and subsequent telomere shortening.

Nardella and Pandolfi, NRC, 2011
Oncogene Addiction, Senescence and the Immune System

Timeline | Important events in senescence research

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1965</td>
<td>Hayflick and Moorhead discover replicative senescence</td>
</tr>
<tr>
<td>1990</td>
<td>Loss of the tumour suppressor PTEN induces premature senescence, termed PICS</td>
</tr>
<tr>
<td>1997</td>
<td>DNA damage identified as a mechanism by which OIS is induced</td>
</tr>
<tr>
<td>2005</td>
<td>Senescence identified as an important component of tumour regression on MYC inactivation</td>
</tr>
<tr>
<td>2006</td>
<td>Senescent cells found to be cleared by the immune system</td>
</tr>
<tr>
<td>2007</td>
<td>The importance of secreted factors in senescence is realized</td>
</tr>
<tr>
<td>2008</td>
<td>Mechanisms of PICS identified</td>
</tr>
<tr>
<td>2010</td>
<td>Telomere erosion identified as being responsible for replicative senescence</td>
</tr>
</tbody>
</table>

OIS, oncogene-induced senescence; PICS, PTEN loss-induced senescence; SKP2, S phase kinase-associated protein 2.

Nardella and Pandolfi, NRC, 2011
Hallmarks of Cancer, Oncogene Addiction and the Immune System

Bendapudi, Rakhra et al CEI, 2011
Oncogene Addiction and the Immune System

Table 1. Examples of immune system-mediated oncogene addiction. Murine models of MYC and BCR-ABL inactivation as well as P53 restoration directly implicate immune involvement in implementing the consequences of oncogene addiction. Below, oncogenes whose tumorigenicity rely heavily on immune evasion and/or pro-tumour inflammation highlight the potentially broad generalizability of the concept of immune system-mediated oncogene addiction.

<table>
<thead>
<tr>
<th>Oncogene</th>
<th>Tumour type</th>
<th>Involved immune compartment</th>
<th>Immune-mediated mechanism</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>MYC</td>
<td>T cell acute lymphoblastic lymphoma</td>
<td>Adaptive immunity (CD4+ T cells)</td>
<td>Induction of senescence and suppression of angiogenesis</td>
<td>[69]</td>
</tr>
<tr>
<td>BCR-ABL</td>
<td>Pro-B cell acute lymphocytic leukaemia</td>
<td>Adaptive immunity (CD4+ T cells)</td>
<td>Induction of senescence and suppression of angiogenesis</td>
<td>[69]</td>
</tr>
<tr>
<td>P53</td>
<td>Hepatocellular carcinoma</td>
<td>Innate immunity (neutrophils, macrophages, NK cells)</td>
<td>Tumour clearance</td>
<td>[82]</td>
</tr>
</tbody>
</table>

Other oncogenic links to tumour immunity

<table>
<thead>
<tr>
<th>Oncogene</th>
<th>Tumour type</th>
<th>Involved immune compartment</th>
<th>Immune-mediated mechanism</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>MYC</td>
<td>B cell lymphoma; pancreatic islet cell tumour</td>
<td>Innate immunity (macrophages; mast cells)</td>
<td>Macrophages induce senescence; mast cells promote angiogenesis</td>
<td>[42,66]</td>
</tr>
<tr>
<td>RAS</td>
<td>Cervical cancer; renal cell carcinoma</td>
<td>Innate immunity (neutrophils)</td>
<td>Neutrophils recruited by IL-8, IL-6 secretion</td>
<td>[58,59]</td>
</tr>
<tr>
<td>MET</td>
<td>Papillary thyroid carcinoma</td>
<td>?Innate immunity/activation of proinflammatory programme</td>
<td>Innate cells recruitment via proinflammatory cytokines/chemokines</td>
<td>[52,57]</td>
</tr>
<tr>
<td>PML</td>
<td>Acute promyelocytic leukaemia; prostate carcinoma</td>
<td>Adaptive immunity (CD8+ T cells)</td>
<td>PML influences MHC class I antigen presentation</td>
<td>[53,54]</td>
</tr>
<tr>
<td>BRAF</td>
<td>Melanoma</td>
<td>Adaptive immunity (dendritic cells, CTLs)</td>
<td>BRAF inhibition up-regulates antigen presentation and decreases IL-10, IL-6</td>
<td>[55,56]</td>
</tr>
</tbody>
</table>

CTL: cytotoxic T lymphocyte; IL: interleukin; MHC: major histocompatibility complex; NK: natural killer.

Bendapudi, Rakhra et al CEI, 2011
Oncogene Addiction and Immune Therapy

A. Mutation 1 → Mutation 2 → Mutation 3 → Remove Mutation 1 or 2 → Apoptosis
   “passenger” or “driver” only in the context of mutations 1 and 2, otherwise deleterious

B. + Oncogenic mutation → + Time → + Inhibitor → Apoptosis

C. Oncogene → senescence/apoptosis

D. Dying tumor cell → Calreticulin, S100's, HMGB1
   → Increased antigen processing, phagocytosis and maturation
   → APC → CD4 T cell → CD8 T cell
   → Cytokines, Chemokines, Anti-Angiogenic factors including TSP-1
   → Induction of senescence → Activation of endogenous response → Killing
   → Immune inhibitors → NK, MDSC

Restifo, Cancer Cell, 2010
p19ARF but not p53 Null Tumors Exhibit a Marked Reduction in Macrophage Infiltration
Pathway Analysis of Gene Expression Identifies Innate Immunity Pathways

Significant Genes
MYC ON/MYC OFF
Fold Change ≥ 2.0
p ≤ 0.05

<table>
<thead>
<tr>
<th>Pathway</th>
<th>MYC</th>
<th>MYC p53-/−</th>
<th>MYC p19-/−</th>
</tr>
</thead>
<tbody>
<tr>
<td>Senescence/Cell Aging Pathways</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Macrophage Activation/Infiltration Pathways</td>
<td>4</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Innate Immune Cell Activation/Infiltration Pathways</td>
<td>4</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Platelet Related Pathways</td>
<td>4</td>
<td>7</td>
<td>3</td>
</tr>
</tbody>
</table>

Pathway Analysis Cut-off - p ≤ 0.05

Adam, Yetil et al., unpublished
Loss of p19ARF Has no Global Effect on MYC Transcription
Loss p19ARF but not p53 Impedes Senescence upon MYC Inactivation

Adam, Yetil et al., unpublished
Loss of p19ARF or p53 Prevents MYC Inactivation from Inducing Sustained Regression

![Survival curve diagram showing the impact of MYC inactivation with or without p19ARF or p53 loss on survival. The graph compares survival rates over time for different groups, with annotations indicating statistical significance with symbols such as * (p<0.001) and ** (p<0.001).]
MYC and a p19ARF Senescence Switch

Alper Yetil PhD  Stacey Adam, PhD
Role of Immune Effectors in Oncogene Addiction

Rakhra et al, Cancer Cell, 2010
Cancer Cell

TUMOR

Remodeling the tumor microenvironment

CD4+ T-cell
BCR-ABL Inactivation Elicits Oncogene Addiction only in an Immune Intact Host
Cyclosporine Blocks Oncogene Addiction

A

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>WT + cyclosporine</th>
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</thead>
<tbody>
<tr>
<td>MYC On</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H&amp;E</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD31</td>
<td></td>
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<tr>
<td>TSP-1</td>
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<td></td>
</tr>
</tbody>
</table>

B

- Ki67
- β-gal
- p16
- p21
- CD31
- TSP-1
Thrombospondins are Required to Elicit Oncogene Addiction upon MYC Inactivation

Lymphocytes must express TSPs

Tumor expression of TSP-1 Bypasses Immune Defect

Rakhra et al, Cancer Cell, 2010
Immune System is Required for “Chemokine Switch” that Contributes to Tumor Regression upon Oncogene Inactivation

Rakhra et al, Cancer Cell, 2010
Immune Effectors Home to Tumor Site upon MYC Inactivation
Reconstitution of CD4+ T-cells Alone Restores Tumor Regression upon MYC Inactivation

**Survival**

- WT
- RAG2+/reconst.CD4+Tcells
- RAG2+/reconst.CD8+Tcells
- RAG2+/CD4

<table>
<thead>
<tr>
<th></th>
<th>RAG2+</th>
<th>RAG2+/CD8+</th>
<th>WT</th>
<th>RAG2+/CD4+</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>**p=0.007</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>*p=0.03</td>
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<td>ns</td>
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</tbody>
</table>

**MRD**

- WT
- RAG2+/reconst.CD4+Tcells
- RAG2+/reconst.CD8+Tcells
- RAG2+/CD4

- **p=0.0002
- **p=0.002
- **p=0.007
- *p=0.03

Rakhra et al, Cancer Cell, 2010
Immune System (CD4+ T-cells) is Required for the Induction of TSP-1 and the Suppression of Angiogenesis

Rakhra et al, Cancer Cell, 2010
Immune System (CD4+ T-cells) is Required for Cellular Senescence upon MYC Inactivation

Rakhra et al, Cancer Cell, 2010
Immune System is Not Required for Proliferative Arrest or Apoptosis upon MYC Inactivation

Rakhra et al, Cancer Cell, 2010
Mechanisms of Tumor Regression upon MYC Inactivation

Tumor: MYC On

Jain et al, Science, 2002
Shachaf et al, Nature, 2004
Giuriato et al, PNAS, 2006
Wu et al, PNAS, 2007
Van Riggelen, Genes and Develop, 2010

MYC Inactivation

Apoptosis

Cellular arrest or Senescence

Differentiation

Inhibition of angiogenesis

Adapted from Felsher D.W (2003)
Immune System is Essential for Sustained Tumor Regression upon MYC Inactivation

Rakhra et al, Cancer Cell, 2010
Immune System is Essential for Sustained Tumor Regression upon MYC Inactivation

Rakhra et al, Cancer Cell, 2010
Immune System, Senescence, Angiogenesis and Oncogene Addiction

Kavya Rakhra
Pavan Bachireddy, MD
Tahera Zabuawaia, PhD
Immune System and Tumorigenesis

Neoplastic progression

Initiation

Pro-inflammatory cytokines

DC migration and antigen presentation

Innate immune response

Serum proteins

Tumour-promoting
- Pro-growth
- Tissue expansion
- Malignant conversion

Cell-death inhibition
- Genomic instability
- Fibroblast activation
- Matrix metabolism
- Angiogenesis

Anti-tumour
- T-cell-mediated cytotoxicity (FAS, perforin and/or cytokine pathways)
- Antibody-dependent cell-mediated cytotoxicity
- Antibody-induced complement-mediated lysis

Secondary lymphoid organs

Adaptive immune response

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Nature Reviews | Cancer

Coussens 2006
Tumorigenesis and Microenvironment

Bissell 2001
Mechanisms of Tumor Regression upon MYC Inactivation

- Apoptosis
- Cellular arrest or Senescence
- Differentiation

Tumor: MYC On

Jain et al, Science, 2002
Shachaf et al, Nature, 2004
Giuriato et al, PNAS, 2006
Wu et al, PNAS, 2007
Van Riggelen, Genes and Develop, 2010

Inhibition of angiogenesis
Adapted from Feilser D.W (2003)
Mechanisms of Oncogene Addiction

**Host Dependent**
- D. Loss of Cues

**Cell Intrinsic**
- E. Differentiation
- F. Arrest
- G. Apoptosis

**Reversing tumorigenesis through restoration of both:**
cell intrinsic fail-safe mechanisms and a normal microenvironment

Restoration of Either p53 or TSP-1 is Sufficient for Tumor Regression Upon MYC Inactivation

Giuriato et al, PNAS, 2007
Oncogene Addiction and the Angiogenic Switch

MYC-regulated p53-dependent TSP-1 Switch

Giuriato, Ryeom, Fan, Bachireddy et al, PNAS, 2007
MYC, Oncogene Addiction and Angiogenesis

Sylvie Giuriato, PhD  Sandra Ryeom, PhD  Alice Fan, MD
Oncogene addiction and senescence

A. No Effect
B. Reversion
C. Partial Loss
D. Loss of Cues

Cell Intrinsic

E. Differentiation
Or SENESCENCE
F. Arrest
G. Apoptosis

Oncogene inactivation restores normal cellular programs that prevent tumorigenesis by resulting in the permanent loss of self-renewal

Senescence a Barrier to Tumorigenesis and Mechanism of Oncogene Addiction

Wu et al PNAS 2007
Loss of p53, RB or p16 Impedes MYC Inactivation from Inducing Cellular Senescence

Lymphoma in vitro
- MYC on
- MYC off

- p53\(^{++/+}\)/MYC
- p53\(^{--/--}\)/MYC
- p53 restored p53\(^{--/--}\)/MYC

Osteosarcoma in vitro
- MYC on
- MYC off

- Empty Vector
- Rb shRNA
- p16INK4a shRNA

Wu et al PNAS 2007
Oncogene Addiction and Senescence
MYC-regulation of self-renewal

beta-galactosidase staining

MYC on

Hepatocellular Carcinoma

MYC off

Lymphoma

Wu et al PNAS 2007
Cellular Senescence and Oncogene Addiction

Natalie Wu  Alper Yetil  Jan van Riggelen
From Mouse to Man

Part II. Mechanism
Oncogene Addiction:
MYC inactivation Induces Differentiation (Senescence)

Cancer specific outcomes to oncogene inactivation

MYC ON
- Lymphoma
- Differentiation/Senescence
- Apoptosis

MYC OFF
- Osteosarcoma
- Differentiation/Senescence
- Bone
- Apoptosis

MYC BACK ON
- Hepatocellular Carcinoma
- Differentiation/Senescence/Apoptosis
- Liver
- Restored Cancer

Felsher et al.
Molecular Cell
(1999)

Jain et al.
Science
(2002)

Shachaf et al.
Nature
(2004)

Tumor Intrinsic Mechanisms of Oncogene Addiction

MYC Inactivation in Lymphoma Results in Arrest, Differentiation and Apoptosis

Giemsa
- None
- Doxycycline

T-Cell

AML

TUNNEL
- None
- Doxycycline

TUNEL

DAPI

Felsher and Bishop, Molecular Cell,
MYC Induced Lymphomagenesis is Reversible

**MYC ON**
- LN-SM
- Thymus
- LN-M
- Spleen
- LN-P

**MYC OFF**

Felsher and Bishop, Molecular Cell,
Conditional Transgenic Mouse Models of Cancer
Cellular functions of MYC

- MYC
- MAX
- CACGTG

- Cell Cycle Regulation
- Apoptosis
- Cell Adhesion
- Differentiation
  - Self-Renewal
- Metabolism
- Protein Synthesis

Adapted from Boxer and Dang, 2001; Perengeris et. al., 2002
Rules of Oncogene Addiction

- Oncogene inactivation reverses tumorigenesis. [Molecular Cell, 1999]
- Brief oncogene inactivation can induce tumor regression. [Science, 2002]
- Nanoscale proteomic analysis of clinical specimens. [Nature Medicine, 2009]
- Autocrine Programs and the Immune System [Genes and Development, 2010; Cancer Cell 2010]
- Modeling Oncogene Addiction [Science Translational Medicine, 2011]
From Mouse to Man
Why do “targeted therapies” work?
Oncogene Addiction: *the Achilles Heel of Cancer*

A. No Effect
B. Reversion
C. Partial Loss
D. Loss of Cues
E. Differentiation
F. Arrest
G. Apoptosis

Felsher and Bishop 1999
Chin and Depinho 1999
Huetner and Tenen 2000
D’Cruz and Chodosh 2001
Fisher and Varmus 2001

Oncogene Addiction: *the* Achilles Heel of Cancer

Addiction to Oncogenes—the Achilles Heal of Cancer

A one-step remedy. Cancer cells acquire abnormalities in multiple oncogenes and tumor suppressor genes (A, B, C, and D). Inactivation of a single critical oncogene (A) can induce cancer cells to differentiate into cells with a normal phenotype or to undergo apoptosis. This dependence on (addiction to) A for maintaining the cancer phenotype provides an Achilles heel for tumors that can be exploited in cancer therapy.

Bernard Weinstein, MD

Science, 2002