CANCER
PROGNOSIS and CHALLENGES

Reeto Basu, PhD
MCB-7200 / 09-20-18
TODAY

- CANCER: Genesis and Progression – 15 mins
- CANCER: Therapy and Challenges – 25 mins
- CANCER: Our Approach – 30 mins
CANCER: Genesis and Progression

- Cancer Stem Cells (CSCs)
- Carcinoma - Sarcoma
- Proto-oncogene
- Tumor suppressor gene
- Metastasis
CANCER: Genesis and Progression

Cellular control of cell growth and proliferation – *seven kinds of mediators*

Proto-oncogene

- Gain-of-function mutation
  - Point mutation
  - Chromosomal translocation
  - Amplification

Viral activation

Oncogene

Tumor suppressor gene

Loss-of-function mutation

Proto-oncogene

Gain-of-function mutation

- Point mutation
- Chromosomal translocation
- Amplification

Viral activation

Oncogene
CANCER: Genesis and Progression

- Proto-oncogene receptor proteins
  - Her2 receptor
  - EGF receptor

  **Gain-of-function**
  - Neu oncoprotein
  - ErbB oncoprotein

- Ligand-independent receptor oncoproteins

  **Loss-of-function**
  - Decreased production of p15 increases proliferation
  - Transcription of gene encoding cell-cycle inhibitor
  - Decreased production of PAI-1 allows increased extracellular matrix degradation and, hence, metastasis
- **CANCER: Genesis and Progression**

**Viral activation**

- Chromosomal translocation

**Constitutive activation**

- Checkpoint failure
CANCER: Genesis and Progression

DNA Pol mediated proof-reading

Spontaneous point mutation: $5\text{-me-C} \rightarrow T$

Chemical mutagens
CANCER: Genesis and Progression

P53: most studied gene in human diseases

- TP53 - tumor protein p53
- Nutrient deprivation
- Hypoxia
- DNA damage
- Oncogene expression
- Ribosomal dysfunction
- Telomere attrition

Senescence

Angiogenesis

Autophagy

Cell-cycle arrest

Apoptosis

Migration

DNA repair

Tumor suppression

ACE - angiotensin I convertin
BRCA1 - breast cancer 1, early

Drosophila melanogaster

Human immunodeficiency virus 1

Damaged DNA

ATM

Chk2

Cdc25A

p53

Transcription activation

Proteasomal degradation

Rapid G1/S block

Apoptosis

Sustained G1 and G2 arrest

DNA repair

P53 inactivating mutations
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Cooperative nature of multiple mutations

Cancer incidence as a function of age
CANCER: Genesis and Progression
Human Liver cancer (Hepatocellular carcinoma/HCC)

- 0.3 - 1 million deaths globally
- Incidence doubled in last 20 years
- Men more likely than women
- Multiple causes (HBV, HCV, alcohol, etc)
- Treatments – surgery, radiation, chemo
Human Pancreatic Cancer
(Pancreatic ductal adenocarcinoma/PDAC)

- 43,000 deaths annually (US)
- 4th in US cancer mortality
- Multiple causes (obesity, smoking, etc)
- Treatments – surgery, radiation, chemo
CANCER: Genesis and Progression
- CANCER: Therapy and Challenges
CANCER: Therapy and Challenges

Targeted therapies
- AZD8055 (mTOR)
- Cetuximab (EGFR)
- Dasatinib (BRAF)
- Dasatinib (BCR-ABL, cKIT, SRC)
- Imatinib (BCR-ABL, cKIT)
- Lapatinib (EGFR, ERBB2/HER2)
- PLX7004 (BRAF)
- Rapamycin (mTOR)
- Ruxolitinib (AKT1 and 2)
- Temsirolimus (mTOR)
- Trastuzumab (ERBB2/HER2)
- Vemurafenib (BRAF)

Radiotherapy
- Single high dose
- Fractionated

Checkpoint inhibitors
- AMP-L24 (PD1)
- Ipilimumab (CTLA-4)
- MPDL3280A (PD-L1)
- Nivolumab (PD1)
- Pembrolizumab (PD1)

Vascular-targeting agents
Anti-angiogenic agents
- Bevacizumab (VEGF)
- DC101 (mVEGFR2)
- Nesvacumab (ANGPT1/2)
- Sunitinib (VEGFRs, PDGFRs, FLT3, CSF1R)
- Sorafenib (VEGFRs, RAF, PDGFRs, cKIT)
- Trebananib (ANGPT1 and 2)

Vascular damaging agents
- Combretastatin A-4 phosphate

Immunomodulatory agents
- AM03100 (CXCR4)
- AM5820 (CSF1R)
- AZD8055 (mTOR)
- BMS-563052 (CXCR2)
- Carfilzomib (CC-122)
- GSK126 (CXCR4)
- IMC-K11 (CSF1R)
- PLX3397 (CD1, CSF1R, FLT3)
- RG7151 (CSF1R)
- SB-567333 (CXCR2)
- SCH527123 (CXCR2)
- S-265610 (CXCR2)

Adaptive immune agents
- AM03100 (CXCR4)
- AZD8055 (mTOR)
- Basiliximab (CD25)
- Blinatumomab (CD3, CD19)
- BMS-663513 (CD37)
- CP-870,893 (CD40)
- Dacaturumab (CD40)
- Dacituzumab (CD25)
- Denileukin diftitox (CD25)
- Lucatumumab (CD40)
- Rapamycin (mTOR)
- Ruxolitinib (CD122)

Chemotherapy
- Antimetabolites
  - 5-Fluorouracil (5-FU)
  - Methotrexate
  - Gemcitabine
- Alkylating agents
  - Cyclophosphamide
  - Daunorubicin
  - Mitoxantrone
- Anthracyclines
  - Doxorubicin
  - Daunorubicin
  - Mitoxantrone
- Platinum compounds
  - Cisplatin
  - Oxaliplatin
- Taxanes
  - Paclitaxel
  - Docetaxel
- Topoisomerase inhibitors
  - Irinotecan
  - Etoposide

Cancer cell

Stromal cell

TRENDS in Immunology
CANCER: Therapy and Challenges

Chemotherapy: DOXORUBICIN

Chemotherapy: GEMCITABINE
▪ CANCER: Therapy and Challenges

Targeted therapy
CANCER: Therapy and Challenges

Immune-therapy: PD1- PD-L1, CTLA4, CAR-T, …
Already a lot of drugs!

...and Drug-resistance
RESISTANCE is natural

- CANCER: Therapy and Challenges

Ref: http://textbookofbacteriology.net/resantimicrobial_3.html
RESEARCH IN CANCER

The Scientist

EXPLORING LIFE, INSPIRING INNOVATION

How Cancers Evolve Drug Resistance

 Researchers unravel the sophisticated ways cancers evade treatments, including immunotherapies, designed to destroy them.

By Anna Azellinsky | April 1, 2017

I’ve been saying this for 15 years: beating cancer takes time, and we need more drugs.

—Charles Sawyers
Memorial Sloan Kettering Cancer Center

PRIMARY RESISTANCE

Within a tumor, genetic diversity exists among cells. Some cells may be resistant to a therapy before they’re ever exposed.

Tumor cells with resistance to drug A

When treated with drug A, only susceptible cells die and the tumor shrinks slightly.

Resistant cells continue to divide and the tumor regrows following treatment.

Eventually, the resistant cells can form new tumors that do not respond to the drug.

ACQUIRED RESISTANCE

Even if none of the cells are initially resistant to a therapy, as they divide the cells acquire genetic mutations that can enable their survival in the face of treatment.

Tumor cells with resistance to drug B

When treated with drug B, most cells die and tumor shrinks significantly. But some cells can acquire resistance.
- CANCER: Therapy and Challenges

MECHANISMS OF RESISTANCE IN CANCER
1. Active drug efflux (ABC-multidrug transporter family)
2. Epithelial-to-Mesenchymal Transition (EMT)
3. Drug-trapping in melanosomes

Ref:
Chen et al., PNAS, 2006;
Chen et al., PCMR, 2009;
Chen et al., JNCI, 2009;
Xie et al., Can.Res., 2009;
Xiao et al., BMC Cancer, 2018
4. Resistance to Immunotherapy

### Table 2. Mechanisms of Primary and Adaptive Resistance to Immunotherapy

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>tumor cell intrinsic</td>
<td>absence of antigenic proteins: low mutational burden, lack of viral antigens, lack of cancer-testis antigens, overlapping surface proteins</td>
</tr>
<tr>
<td></td>
<td>absence of antigen presentation: deletion in TAP, deletion in B2M, silenced HLA</td>
</tr>
<tr>
<td></td>
<td>genetic T cell exclusion: MAPK oncopgenic signaling, stabilized b-catenin, mesenchymal transcriptome, oncopgenic PD-L1 expression</td>
</tr>
<tr>
<td>insensibility to T cells</td>
<td>mutations in interferon gamma pathway signaling</td>
</tr>
<tr>
<td>tumor cell extrinsic</td>
<td>absence of T cells: lack of T cells with tumor antigen-specific TCRs</td>
</tr>
<tr>
<td></td>
<td>inhibitory immune checkpoints: VISTA, LAG-3, TIM-3</td>
</tr>
<tr>
<td></td>
<td>immunosuppressive cells: TAMs, Tregs</td>
</tr>
</tbody>
</table>

Resistance to cancer immunotherapy mediated by apoptosis of tumor-infiltrating lymphocytes

Jingjing Zhu\(^1\,2\,3\), Céline G. Powis de Tenbosch\(^1\,2\), Stefania Cané\(^1\,2\,3\), Didier Colau\(^1\,2\), Nicolas van Baren\(^1\,2\), Christophe Lurquin\(^1\,2\), Anne-Marie Schmitt-Verhulst\(^4\), Peter Lijestrom\(^5\), Catherine Uyttenhove\(^1\,2\) & Benoit J. Van den Eynde\(^1\,2\,3\)

Leading Edge Review

Primary, Adaptive, and Acquired Resistance to Cancer Immunotherapy

Padmanee Sharma\(^1\,2\), Siwen Hu-Lieskov\(^3\), Jennifer A. Wargo\(^3\), and Antoni Ribas\(^2\,4\)

*Department of Genitourinary Medical Oncology and Immunology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA
*Department of Medicine, Division of Hematology-Oncology, University of California, Los Angeles and the Jonsson Comprehensive Cancer Center, Los Angeles, CA 90095, USA
*Department of Melanoma Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA
*Correspondence: padmanee@mdanderson.org (P.S.), arribas@mednet.ucla.edu (A.R.)

http://dx.doi.org/10.1016/j.cell.2017.01.017
The big question is whether it's the tumor cells that are becoming resistant, if the immune system is becoming dysfunctional, or a combination of both.

—Jesse Zaretsky
University of California, Los Angeles
GROWTH HORMONE (GH) AXIS IN CANCER

- CANCER: Our Approach
Acromegaly (gigantism)  normal  growth deficit

GH-GHR axis
GH-GHR axis – Growth, Metabolism, Aging
Hundreds of *in vitro* and *in vivo* studies have described elevated expression of GH or GHR or both in 12 different cancer types (Basu, Qian, Kopchick, EJE, 2018)
Cancer incidence correlates with GH action in human population

IGF-I deficiency, longevity and cancer protection of patients with Laron syndrome

Zvi Laron a, Rivka Kauli a, Lena Lapkina b, Haim Werner b

Published in final edited form as:

Growth Hormone Receptor Deficiency is Associated With a Major Reduction in Pro-aging Signaling, Cancer and Diabetes in Humans

Jaime Guevara-Aguirre1, Priya Balasubramanian2,4, Marco Guevara-Aguirre1, Min Wei4, Federica Madia4, Chia-Wei Cheng4, David Hwang5, Alejandro Martin-Montalvo6,7, Jannette Saavedra1, Sue Ingles8, Rafael de Cabo6, Pinchas Cohen9, and Valter D. Longo2,3,4

1 June 2018, Pages 2182–2188, https://doi.org/10.1210/jc.2017-02457

Published: 23 March 2018 Article history
GH-GHR action - Human melanoma – Therapy resistance

Ref: Sustarsic et al., 2013
Analyses of Intracellular Signaling
In human melanoma, GH-GHR signaling directly activates JAK2/STAT5, STAT3, STAT1, SRC, AKT and mTOR ERK1/2
GH-GHR signaling directly activates JAK2/STAT5, STAT3, SRC and ERK1/2 in human HCC
GH-GHR action - Drug sequestration in melanosomes

Ref: Basu et al., manuscript under review, 2018
GH-GHR action - Drug sequestration in melanosomes

Ref: Basu et al., manuscript under review, 2018
TCGA data – Correlation with *in vitro* observations

Ref: Basu et al., *manuscript under review*, 2018
GH-GHR action – EMT

Ref: Basu et al., Oncotarget, 2017a

Ref: Qian et al., unpublished data; 2017
GH-GHR action - upregulates drug efflux via ABC transporters

With 2.5 nM GH + GHR knockdown

Protein Levels following GHRKO

Drug Retention Assay
Autocrine GH-GHR action - Drug resistance and EMT markers

Ref: Basu et al., under review, 2018
TCGA data – Correlation with *in vitro* observations

**male melanoma (TCGA set) - ABC efflux pump expression**

- **low-GHR**
- **high-GHR**

**female melanoma (TCGA set) - ABC efflux pump expression**

- **low-GHR**
- **high-GHR**

Ref: Basu et al., *manuscript under review*, 2018
GH-GHR action – ECM remodeling – TGFβ, collagen, MMPs

Roles of TGFβ in cancer

Premalignant state
- Tumor-suppressive effects: cytostasis, differentiation, apoptosis
- Suppression of tumorigenic inflammation
- Suppression of stroma-derived mitogens

Malignant progression (Loss of tumor suppression)
- Evasion of immune surveillance
- Autocrine mitogen production
- Motility

Invasiveness and dissemination
- Epithelial-mesenchymal transition
- Myofibroblast mobilization
- Cancer cell priming for metastasis

Metastatic colonization
- Extravasation
- Osteoclast mobilization
- Microenvironmental-modifying factors: cytokines, proteases

Ref: Massague, Cell, 2008
GH-GHR action – ECM remodeling

Ref: Basu et al., under review, 2018
TCGA data analyses: low GHR vs. high GHR - melanoma

**melanoma (TCGA set) - sex distribution**

<table>
<thead>
<tr>
<th></th>
<th>low-GHR</th>
<th>high-GHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>63</td>
<td>61</td>
</tr>
<tr>
<td>Female</td>
<td>37</td>
<td>39</td>
</tr>
</tbody>
</table>

**melanoma (TCGA set) - sex-specific % metastasis**

<table>
<thead>
<tr>
<th></th>
<th>low-GHR</th>
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<tbody>
<tr>
<td>Male</td>
<td>75</td>
<td>93</td>
</tr>
<tr>
<td>Female</td>
<td>74</td>
<td>85</td>
</tr>
</tbody>
</table>

Ref: Basu, Kruse, Kopchick., *unpublished data*, 2018
TCGA data analyses: low GHR vs. high GHR - melanoma

Ref: Basu, Kruse, Kopchick., *unpublished data*, 2018
GHR-antagonist treatment → combination therapy

- GHR-antagonist Pegvisomant alone was successful in significantly reducing tumor burden in xenograft mouse models in cancers of breast (Perry et al., 2011, Bougen et al., 2012), prostate (Recouvreux et al., 2017), colon (Dagnaes-Hansen et al, 2004), meningioma (Friend et al, 1999; McCutcheon et al, 2001), and endometrium (Pandey et al., 2008).

Ref: Basu et al., Hormones and Cancer, 2017b
GHR Antagonist (Somavert)

Amino Acid Sequence of Pegvisomant Protein
Blocking GHR blocks chemoresistance

C = DMSO  
CGH = DMSO + 20ng/mL GH  
V = 20nM vemurafenib  
VGH = 20nM vemurafenib + 20ng/mL GH

<table>
<thead>
<tr>
<th>sample</th>
<th>C</th>
<th>CGH</th>
<th>V</th>
<th>VGH</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC50-</td>
<td>1.0</td>
<td>4.9</td>
<td>11.8</td>
<td>25.3</td>
</tr>
<tr>
<td>ratio</td>
<td>(9nM)</td>
<td>(38nM)</td>
<td>(139nM)</td>
<td>(278nM)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>sample</th>
<th>C</th>
<th>CGH</th>
<th>V</th>
<th>VGH</th>
</tr>
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<tbody>
<tr>
<td>EC50-</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ratio</td>
<td>(10.2nM)</td>
<td>2.1</td>
<td>6.4</td>
<td>8.1</td>
</tr>
<tr>
<td></td>
<td>(24.6nM)</td>
<td></td>
<td>(75.3nM)</td>
<td>(98.8nM)</td>
</tr>
</tbody>
</table>
EC50 shift – doxorubicin:

HepG2 – doxorubicin – EC50 [cell viability]

Blocking GHR lowers EC50 dose of doxorubicin in HepG2

<table>
<thead>
<tr>
<th>Condition</th>
<th>EC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>557 nM</td>
</tr>
<tr>
<td>+GHA</td>
<td>245 nM</td>
</tr>
<tr>
<td>+GH</td>
<td>544 nM</td>
</tr>
<tr>
<td>+GH+GHA</td>
<td>229 nM</td>
</tr>
</tbody>
</table>
Blocking GHR sensitizes GH-responsive human cancers to anti-cancer treatments by attenuating multiple mechanisms of drug efflux, drug sequestration, ECM remodeling, mitochondrial function, and EMT.

Combining GHR antagonism with present / developing anti-cancer therapy can lead to more efficacious targeting of therapy resistant human cancers.

SUMMARY

EC50 of xxx-x9 alone = 1500 nM
EC50 of xxx-x9 + siGHR = 22 nM

Ref: Basu et al., unpublished data, 2017