CANCER
PROGNOSIS and CHALLENGES

Reeto Basu, PhD
MCB-7200 / 09-19-19
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
- Oncogene
- Tumor suppressor gene
  - *loss-of-function mutation*
- Viral activation
CANCER: Genesis and Progression

- **gain-of-function**
- **loss-of-function**
CANCER: Genesis and Progression

- Chromosomal translocation
- Constitutive activation
- Viral activation
- Checkpoint failure
CANCER: Genesis and Progression

DNA Pol mediated proof-reading

Spontaneous point mutation: 5-me-C → T

Chemical mutagens

- Afatoxin B
- Benzo(a)pyrene (3,4-benzo(a)pyrene)
**CANCER: Genesis and Progression**

**P53: most studied gene in human diseases**

- TP53 - tumor protein p53
- # of citations: 6622

Diagram showing the role of p53 in various cellular processes such as senescence, angiogenesis, autophagy, apoptosis, cell-cycle arrest, migration, and DNA repair.

**Tumor suppression**

- ACE - angiotensin I converting enzyme 2137
- BRCA1 - breast cancer 1, early 2087
- Drosophila melanogaster
- Human immunodeficiency virus 1

**P53 inactivating mutations**

Diagram illustrating the impact of P53 inactivation on DNA repair and cell cycle control.

- Damaged DNA -> ATM -> Chk2 -> p53 stabilization
- p53 stabilized -> Transcription activation
- p53 stabilized -> Proteasomal degradation
- p53 stabilized -> Rapid G1/S block
- p53 stabilized -> Apoptosis
- p53 stabilized -> Sustained G1 and G2 arrest
- p53 stabilized -> DNA repair
CANCER: Genesis and Progression

- Cooperative nature of multiple mutations
- Cancer incidence as a function of age
CANCER: Genesis and Progression

(a) (b)

[Images of tissue samples showing differentiation]

transformation

tumor cells

normal cells
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)
- Dabrafenib (BRAF)
- Dasatinib (BCR-ABL, cKIT, SRC)
- Imatinib (BCR-ABL, cKIT)
- Lapatinib (EGFR, ERBB2/HER2)
- PLX4720 (BRAF)
- Rapamycin (mTOR)
- Rituximab (CD20)
- Ruxolitinib (JAK1 and 2)
- Temsirolimus (mTOR)
- Trastuzumab (ERBB2/HER2)
- Vemurafenib (BRAF)

Radiotherapy
- Single high dose
- Fractionated

Immunomodulatory agents
Innate immune cells
- AMG3100 (EXC4)
- AMG3820 (CSF1R)
- AZD8369 (CXR2)
- BLZ945 (CSF1R)
- Carfilzomib (CCL2)
- GSK1257656 (CXR2)
- INCB018480 (CSF1R)
- PLX3397 (cKIT, CSF1R, FLT3)
- RG7155 (CSF1R)
- SB-656933 (CXR2)
- SCH527123 (CXR2)
- S-265610 (CXR2)
- Trabectedin

Adaptive immune cells
- AMG3100 (EXC4)
- AZD8369 (mTOR)
- Bafetinib (PDGFR)
- Bortezomib (CD38)
- Blinatumomab (CD3, CD19)
- BMS-663513 (CD137)
- CP-870,893 (CD40)
- Dactinomycin (CD40)
- Dacarbazine (CD25)
- Denileukin diftitox (CD25)
- Eculizumab (CD40)
- Ectolin (mTOR)
- Rapamycin (mTOR)
- Rituximab (CD20)
- Temsirolimus (mTOR)

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

Checkpoint inhibitors
- Anti-angiogenic agents
  - Bevacizumab (VEGFA)
  - DC101 (sVEGFR2)
  - Nivolumab (ANGPT1)
  - Sorafenib (VEGFRs, PDGFRs, FLT3, CSF1R)
  - Trabectedin (ANGPT1 and 2)

Vascular damaging agents
- Combretastatin A-4 phosphate

TRENDS in Immunology
CANCER: Therapy and Challenges

Chemotherapy: DOXORUBICIN

Chemotherapy: GEMCITABINE
CANCER: Therapy and Challenges

Targeted therapy

- Cetuximab
- Panitumumab
- Trastuzumab
- Pertuzumab
- TDM1
- Lapatinib
- Neratinib
- Afatinib
- MEHD7945A (dual-action Ab targeting EGFR and ERBB3)
- MEHD7945A (dual-action Ab targeting EGFR and ERBB3)
- MM-121
- AMG-888
- 17-AAG and PI-504
- GSK118436 and PLX4032
- GSK120212 and AZD6244
- PI3K inhibitors (e.g., GDC-0941 and PH-896)
- AKT inhibitors (e.g., MK208 and GDC-0068)
- mTOR inhibitors (e.g., rapamycin and INK128)
CANCER: Therapy and Challenges

Immune-therapy: PD1- PD-L1, CTLA4, CAR-T, …

Cancer immunotherapy targeting the CD47/SIRPα axis.

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In vivo CRISPR screening identifies Ptpn2 as a cancer immunotherapy target.

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Already a lot of drugs!

...and Drug-resistance
RESISTANCE is natural

Ref: http://textbookofbacteriology.net/resantimicrobial_3.html

▪ CANCER: Therapy and Challenges
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.

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 A

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

When treated with drug B, most cells die and tumor shrinks significantly. But some cells can acquire resistance.

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

- EMT signals at tumor margin
- Partial EMT state facilitates motility and invasion into stroma
- Quasi-mesenchymal cell
- Quasi-epithelial cell
- Epithelial cell

- 3. Mesenchymal phenotype facilitates intravasation and anoikis resistance during dissemination and extravasation
- 4. Migrating cancer stem cell
- 5. Survival and dormancy at distant site
- 6. Exit from dormancy and early colonization
- 7. Colonization: proliferation and formation of macrometastasis

- CANCER: Therapy and Challenges
CANCER: Therapy and Challenges

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>
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<tbody>
<tr>
<td>tumor cell intrinsic</td>
<td>absence of antigen proteins, low mutational burden, lack of viral antigens, lack of cancer-testis antigens, overlapping surface proteins</td>
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<td>absence of antigen presentation, deletion in TAP, deletion in B2M, silenced HLA</td>
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<td>genetic T cell exclusion, MAPK oncogenic signaling, stabilized b-catenin, mesenchymal transcriptome oncogenic PD-L1 expression</td>
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<td>insensitivity to T cells, mutations in interferon gamma pathway signaling</td>
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<tr>
<td>tumor cell extrinsic</td>
<td>absence of T cells, lack of T cells with tumor antigen-specific TCRs</td>
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<td>inhibitory immune checkpoints, VISTA, LAG-3, TIM-3</td>
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<td>immunosuppressive cells, TAMs, Tregs</td>
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Resistance to cancer immunotherapy mediated by apoptosis of tumor-infiltrating lymphocytes

Jingjing Zhu1,2,3, Céline G. Powis de Tenbosch1,2, Stefania Cané1,2,3, Didier Colau1,2, Nicolas van Baren1,2, Christophe Lurquin1,2, Anne-Marie Schmitt-Verhulst4, Peter Ljieström5, Catherine Uyttenhove1,2, & Benoit J. Van den Eynde1,2,3

Primary, Adaptive, and Acquired Resistance to Cancer Immunotherapy

Padmanee Sharma,1,4 Siwen Hu-Lieskovsk1,2 Jennifer A. Wargo,3 and Antoni Ribas2,5
1Department of Genitourinary Medical Oncology and Immunology, The University of Texas MD Anderson Cancer Center, Houston, TX, 77030, USA
2Department of Medicine, Division of Hematology-Oncology, University of California, Los Angeles and the Jonsson Comprehensive Cancer Center, Los Angeles, CA 90095, USA
3Department of Melanoma Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, 77030, USA
4Correspondence: padsharma@mdanderson.org (P.S.), aribas@mednet.ucla.edu (A.R.)
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
CANCER: Our Approach

GROWTH HORMONE (GH) AXIS IN CANCER
GH-GHR axis

Acromegaly (gigantism)  normal  growth deficit
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

Mutation Research/Reviews in Mutation Research
Volume 772, April–June 2017, Pages 123-133

Review
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

Colorectal cancer – Survival Analysis

Colorectal cancer – GHR immunostaining

Published SIR was higher in single-center studies and in studies with <10 cancer cases.
GH-GHR action - Human melanoma – Therapy resistance

Ref: Sustarsic et al., 2013
Analyses of Intracellular Signaling
GH-GHR
Intracellular Signaling

Image: Fernandez-Perez et al., 2013, Frontiers in Endocrinology
In human melanoma, GH-GHR signaling directly activates JAK2/STAT5, STAT3, STAT1, SRC, AKT and mTOR, ERK1/2.
1. Epithelial-to-Mesenchymal Transition (EMT)
Targeting growth hormone receptor in human melanoma cells attenuates tumor progression and epithelial mesenchymal transition via suppression of multiple oncogenic pathways.

Basu R1,2, Wu S1,2, Knopick JJ1,2,3.

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2 Molecular and Cell Biology Program, Ohio University, Athens, Ohio, USA.
3 Ohio University Heritage College of Osteopathic Medicine, Athens, Ohio, USA.

Abstract
Recent reports have confirmed highest levels of growth hormone (GH) receptor (GHR) transcripts in melanoma, one of the most aggressive forms of human cancer. Yet the mechanism of GH action in melanoma remains mostly unknown. Here, using human malignant melanoma cells, we examined the effects of GH excess or siRNA mediated GHR knock-down (GHRKD) on tumor proliferation, migration and invasion. GH promoted melanoma progression while GHRKD attenuated the same. Western blot analysis revealed drastic modulation of multiple oncogenic signaling pathways (JAK2, STAT1, STAT3, STAT5, AKT, mTOR, SRC and ERK1/2) following addition of GH or GHRKD. Further, we show that GH excess upregulates expression of markers of epithelial mesenchymal transition in human melanoma, while the effects were reversed by GHRKD. Interestingly, we observed consistent expression of GH transcript in the melanoma cells as well as marked modulation of the IGF receptors and binding proteins (IGF1R, IGF2R, IR, IGFBP2, IGFBP3) and the oncogenic HGF-MET mRNA, in response to excess GH or GHRKD. Our study thus identifies the mechanistic model of GH-GHR action in human melanoma and validates it as an important pharmacological target of intervention.

KEYWORDS: IGF-1; cancer; growth hormone (GH); growth hormone receptor (GHR); melanoma

PMID: 26223541 PMCID: PMC5400500 DOI: 10.18632/oncotarget.15375
- GH treatment increases EMT marker expression
- siRNA-mediated GHRKD suppresses EMT marker expression

Basu et al, 2017, Oncotarget
2. Active drug efflux (ABC-multidrug transporter family)

Basu R1,2, Baumgaertel N1,3, Wu S1,2, Kopchick JJ4,6,6

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6 Heritage College of Osteopathic Medicine, Athens, OH, USA. kopchick@ohio.edu.

Abstract
Melanoma remains one of the most therapy-resistant forms of human cancer despite recent introductions of highly efficacious targeted therapies. The intrinsic therapy resistance of human melanoma is largely due to abundant expression of a repertoire of xenobiotic efflux pumps of the ATP-binding cassette (ABC) transporter family. Here, we report that GH action is a key mediator of chemotherapeutic resistance in human melanoma cells. We investigated multiple ABC efflux pumps (ABCB1, ABCB5, ABCB8, ABCC1, ABCC2, ABCG1, and ABCG2) reportedly associated with melanoma drug resistance in different human melanoma cells and tested the efficacy of five different anti-cancer compounds (cisplatin, doxorubicin, ortonin, paclitaxel, vemurafenib) with decreased GH action. We found that treatment of human melanoma cells upregulates expression of multiple ABC transporters and increases the EC50 of melanoma drug vemurafenib. Also, vemurafenib-resistant melanoma cells had upregulated levels of GH receptor (GHR) expression as well as ABC efflux pumps. GHR knockdown (KD) using siRNA in human melanoma cells treated with sub-EC50 doses of anti-tumor compounds resulted in significantly increased drug retention, decreased cell proliferation and increased drug efficacy, compared to mock-transfected controls. Our set of findings identify an unknown mechanism of GH regulation in mediating melanoma drug resistance and validates GHR as a unique therapeutic target for sensitizing highly therapy-resistant human melanoma cells to lower doses of anti-cancer drugs.
GH-GHR action - upregulates drug efflux via ABC transporters

With 2.5 nM GH + GHR knockdown

Protein Levels following GHRKO

Baseline: 0, 2-fold change: 1, 4-fold change: 2, 8-fold change: 3

Ref: Basu et al., Hormones and Cancer, 2017b

Drug Retention Assay

Ref: Qian et al., unpublished data; 2017

Basu et al, 2017, Hormones and Cancer
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*, 2019
GH-GHR action – ECM remodeling – TGFβ, collagen, MMPs

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

Ref: Basu et al., unpublished data
GHR-antagonist treatment → combination therapy

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<th>scr-RNAi + drug (DAPI)</th>
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Ref: Basu et al., Hormones and Cancer, 2017b
GHR Antagonist
(Somavert)

Amino Acid Sequence of Pegvisomant Protein
Blocking GHR blocks chemoresistance

Ref: Basu et al., Hormones and Cancer, 2017b
 Blocking GHR lowers EC50 dose of doxorubicin in HepG2

Ref: Basu et al., unpublished
 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**

![Diagram of cancer cell mechanisms](image)

- **DRUG RESISTANT CANCER CELL**
- **DRUG SENSITIVE CANCER CELL**

- **log-dose vs response**
  - EC50 of xxx-x9 alone = 1500 nM
  - EC50 of xxx-x9 + siGHR = 22 nM

Ref: Basu et al., *unpublished data, 2017*
THANK YOU!

Kopchick Lab

Edison Biotechnology Institute

Emily Davis

Diego Ibarra