Convergent Evolution and the Origins of Lethal Cancer

Kenneth J. Pienta, MD
Donald S. Coffey Professor of Urology
Professor of Urology, Oncology, Pharmacology and Molecular Sciences, and Chemical and Biomolecular Engineering, Johns Hopkins University
Director, Brady Urologic Research Institute
Cancer is an ongoing health crisis.

Cancer kills 10 million people a year globally.

In the United States, 600,000 people are dying every year from cancer.

1 person is dying every minute.
Cancer kills people for two reasons:

• It spreads to all parts of the body (metastasis).

• It is resistant to all known forms of systemic treatment.
  • Cancer is only routinely cured if it can be cut out or killed with focused radiation.
    • Traditionally, this has been explained by the thought that within the billions of cancer cells in a tumor, resistance to therapies evolves by random chance that endows at least one cancer cell with resistance to any particular therapy.
  • This explanation relies on chance since lethal cancer demonstrates resistance to therapeutic agents that it has previously not been exposed to.
Metastatic Cancer is ultimately resistant to virtually all systemic therapies.

• From an evolutionary ecology perspective – these two processes, both requiring resiliency and the same co-adaptations, are likely linked.
  • Cancer arises independently and is lethal in 10 million people per year
  • We believe that the **lethality** resulting from metastasis and resistance an example of *convergent evolution*. 
20 million cancer diagnoses

10 million cancer patients cured with local therapy

10 million cancer patients progress with metastatic and therapy resistant disease

1 convergent lethal cancer phenotype
Convergent evolution

- **Convergent evolution** is the independent evolution of similar features across species of different periods or epochs in time. Convergent evolution creates *analogous structures* that have similar form or function but were not present in the last common ancestor of those groups.
  - Wings
  - Hooves
  - Teeth
  - Eyes
Evolutionary clades

A clade is a monophyletic group derived from a common ancestor and including all its lineal descendants.

We need to understand why this results in lethality
Classically, metastasis and resistance are considered two distinct processes, attributed to tumor heterogeneity but studied by different groups of scientists.
Can we explain metastasis and resistance within a single silo of study?
Modeling of metastasis and resistance by creating the “cancer swamp”

Generate, maintain and reverse gradients

Drugs
Nutrients
Cytokines

Inlet A
Outlet A
Outlet B
Inlet B

Microfluidics Array

Fluorescein Gradient
Automated tracking

PC3 prostate cancer cells in lethal chemotherapy

Low docetaxel

14 hours

High docetaxel
PC3 prostate cancer cells in lethal chemotherapy
Polyaneuploid cells can asymmetrically divide and generate $2N^+$ cells (“bloom”)  

A. Aneuploid  
B. Polyploid  

1. Highly motile  
2. Highly resistant  
3. Seed recurrence
PACCs first described in 1858
Rudolf Virchow (Father of Modern Pathology)

1858: Cellular Pathology as based upon Physiological and Pathological Histology

Fig. 142
Various, polymorphous cancer-cells, some of them in a state of fatty degeneration, two with multiplication of nuclei. 300 diameters.

Multinucleated polyploid cells have been reported in the literature.

The formation of giant multinucleated polyploid cells after therapeutic intervention has been well described:
- Chemotherapy
- Radiotherapy
- Tumor microenvironment

It has been assumed by the majority of the cancer community that these giant polyploid cells do not survive and die due to mitotic catastrophe subsequent to multipolar cell division or simply senesce.
More silo’ed research

Polyaneuploid cancer cells (PACCs) are central actuators of tumorigenesis, metastasis, and therapeutic resistance.
PACCs are found in multiple cancer types

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>Cell lines / % HACCs</th>
<th>Histopath</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>MDA-MB-231: 2% MCF7: 3%</td>
<td>![Histopath]</td>
</tr>
<tr>
<td>Colon</td>
<td>CACO-2: 8% HCT116: 2%</td>
<td>![Histopath]</td>
</tr>
<tr>
<td>Ovarian</td>
<td>SKOV3: 14% HEY-T30: 2%</td>
<td>![Histopath]</td>
</tr>
<tr>
<td>Lung</td>
<td>H2126: 2% H2087: 8%</td>
<td>![Histopath]</td>
</tr>
<tr>
<td>Glioblastoma</td>
<td>DIPG-JHU-1: 2% BT94: 4% U138-MG: 2%</td>
<td>![Histopath]</td>
</tr>
<tr>
<td>Bladder</td>
<td></td>
<td>![Histopath]</td>
</tr>
</tbody>
</table>
We know that PACCs are relevant to human cancer (not just cells grown in a lab).

What have we learned about PACCs?
How have we missed these cells for so long?

Dose response curve: PC3/docetaxel

We have trained our eye to ignore them (or we don’t look at the cells at all!)
PACCs are physically larger and have more DNA than “typical” parent cancer cells.
PACCs are morphologically distinct and have irregular nuclei
More PACCs are formed after treatment, regardless of therapy type of cancer cell line

<table>
<thead>
<tr>
<th>%PACCs after 72h treatment</th>
<th>PC3</th>
<th>DU145</th>
<th>LNCaP</th>
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<tbody>
<tr>
<td><strong>Docetaxel</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 nM</td>
<td>3.9%</td>
<td>2.7%</td>
<td>2.9%</td>
</tr>
<tr>
<td>0.1 nM</td>
<td>10.2%</td>
<td>19.2%</td>
<td>4.75%</td>
</tr>
<tr>
<td>1 nM</td>
<td>13.7%</td>
<td>43.4%</td>
<td>32.9%</td>
</tr>
<tr>
<td>5 nM</td>
<td>35.3%</td>
<td>44.8%</td>
<td>94.8%</td>
</tr>
<tr>
<td><strong>Etoposide</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 uM</td>
<td>3.2%</td>
<td>3.5%</td>
<td>2.7%</td>
</tr>
<tr>
<td>2 uM</td>
<td>12.3%</td>
<td>82.9%</td>
<td>60.5%</td>
</tr>
<tr>
<td>16 uM</td>
<td>18.7%</td>
<td>82.0%</td>
<td>64.9%</td>
</tr>
<tr>
<td>50 uM</td>
<td>22.3%</td>
<td>80.1%</td>
<td>78.9%</td>
</tr>
<tr>
<td><strong>Cisplatin</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 uM</td>
<td>3.2%</td>
<td>3.6%</td>
<td>3.1%</td>
</tr>
<tr>
<td>0.6 uM</td>
<td>4.2%</td>
<td>31.9%</td>
<td>6.3%</td>
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<tr>
<td>5 uM</td>
<td>4.6%</td>
<td>71.8%</td>
<td>32.5%</td>
</tr>
<tr>
<td>16 uM</td>
<td>10.8%</td>
<td>76.6%</td>
<td>67.1%</td>
</tr>
</tbody>
</table>
Dramatic increase in the **total number** of PACCs after therapy

Seed $1 \times 10^6$ cells ~30,000 PACCs

Tx 72h

Harvest ~300,000 PACCs
PACCs formation: may be formed by multiple mechanisms (late endomitosis)
PACCs are live, active, and functional cells with increased motility.

To our knowledge, we are among the first to intentionally image and track PACCs. Most drug testing is with plate-based assays.

![Graph showing distance moved (µM) compared to typical PGCC](image)

George Butler

MDA-MB-231 breast cancer cells
PC3-FUCCI cells were cultured with [LD90] docetaxel for 72 hours.

>90% of the surviving cells that continued to persist for 12 days were in G1/G0.
PACCs have increased fat stores: lipid droplets

PACCs likely use these fat stores to survive while stress is present

NileRed stain: GREEN stain = lipid droplets
PACCs have a distinct mRNA profile

Metastasis
Therapy resistance

parental  PACCs
PACCs are resilient cells that
1) form in response to stress
2) survive therapy
3) are highly motile

KEY ATTRIBUTES:
1) Whole genome doubling - polyploid and aneuploid
2) Exit from the cell cycle - quiescence

How are can this be explained?
The ability to access polyploid programs enables the formation of PACCs.
Table 1. Postulated Consequences for Polyploidization

Genomics:

1. **Increased genomic stability.** Extra copies of genes allow organisms to avoid lethal genomic damage, e.g., preventing Muller’s ratchet in protists.
2. **Increased heritable variation.** The increased genomic material allows increased mutation in response to stress. Genetic instability creates progeny of various fitness allowing selection of a robust clone, e.g., antibiotic resistance in some yeast strains.
3. **Self-genetic modification.** Increased genomic material provides self-genetic modification through directed reprogramming, e.g., antibiotic resistance in some bacteria strains.
4. **New functionality.** Redundant genomic material allows mutation to achieve a new functionality. For example, two pairs of limbs allows one pair to become wings.

Function:

5. **Induction of quiescence.** Halting of the cell cycle leads to a non-proliferative state as a mechanism to protect the non-dividing genome while stress is present, e.g. *Entamoeba histolytica.*
6. **Increased storage capacity.** Increased cell size increases storage capacity needed for sustained quiescence (genomic material is a passenger), e.g., plant vacuoles.
7. **Increased cell function.** Increased cell size increases cell function (genomic material is a passenger), e.g., osteoclast fusion for the production of acid to lyse bone.
8. **Increased metabolic capacity.** Increased gene dosage increases production of RNA and protein products necessary for increased cell metabolism for growth, e.g., megakaryocytes.
9. **Increased toxin protection.** Increased gene dosage increases production of RNA and protein products necessary to protect from oxidative damage and cell size may protect from short term environmental toxic stresses, e.g., hepatocytes.
STRESS

Tumor cell heterogeneity model

Mutation present

Clonal outgrowth

Quiescent state model

No mutation

Resistant clonal outgrowth

Increased genomic material is a passenger: the quiescent state is the resistant clone.

Evolutionary triage model

Mutation selected

Resistant clonal outgrowth

Increased genomic material allows random rearrangements to find resistant clone.

Self genetic modification model

Mutation generated

Resistant clonal outgrowth

Increased genomic material allows directed rearrangement to generate the resistant clone.

PACC formation is an obligate step of the resistance program of randomly generated and already present clones.
PACCs can give rise to a “recurrence” of typical-sized cells

PACCs induced w/ Cisplatin

recurrence

75 days

Metastasis
Therapy resistance
PACCs can give rise to a “recurrent” population with typical DNA amounts

Figure 3. PGCCs express stem-like markers. >4N+ PGCCs and 2N+ cells were isolated by FACS and expression assessed by RT-qPCR.

DNA content

Cancer stem cell panel

- 2N+
- >4N+

<table>
<thead>
<tr>
<th>Marker</th>
<th>2N+</th>
<th>&gt;4N+</th>
</tr>
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<tbody>
<tr>
<td>CD24</td>
<td>41.3</td>
<td></td>
</tr>
<tr>
<td>CD44</td>
<td>14.8</td>
<td></td>
</tr>
<tr>
<td>CD133</td>
<td>270.6</td>
<td></td>
</tr>
<tr>
<td>KLF4</td>
<td>160.1</td>
<td></td>
</tr>
<tr>
<td>MYC</td>
<td>170.4</td>
<td></td>
</tr>
<tr>
<td>NANOG</td>
<td>163.6</td>
<td></td>
</tr>
<tr>
<td>OCT4</td>
<td>206.2</td>
<td></td>
</tr>
<tr>
<td>SOX2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Relative expression (2N+ = 1)

parental

+ Tx

PACCs

recurrent population
PACCs may repopulate through multiple mechanisms

- Neosis
- Asymmetric division

Divide from PACC to typical size and DNA amount
Polyaneuploid Cancer Cell
(paused proliferation, motile)

Therapy

Recurrence

Dormant disseminated tumor cells

Metastatic cascade

Reactivation and proliferation

METASTASIS  RESISTANCE

CONVERGENT LETHAL PHENOTYPE
THERAPEUTIC RESISTANCE through PACCs is a HALLMARK of LETHAL CANCER

Hallmarks of Cancer
- sustaining proliferative signaling
- evading growth suppressors
- resisting cell death
- enabling replicative immortality
- inducing angiogenesis
- activating invasion and metastasis
- avoiding immune destruction
- deregulating cellular energetics

Enabled by:
- genome instability and mutation
- tumor promoting inflammation

Hallmarks of Lethal Cancer
- therapeutic resistance

Enabled by:
- polyploidization
- reversible cell cycle arrest
Cancer evolves resistance to all known therapy

We believe that this resistance is achieved through a “PACC” phase.
Evolutionary double bind treatment strategy for cancer cure

1. Treat with cytotoxic therapy to kill the majority of the cancer cell population AND induce PACCs.
2. Treat with a therapy that eradicates the PACCs.
Apply an evolutionary double bind to cure cancer through PACC-directed therapy

1. Use traditional anti-cancer therapy to induce evolution of PACCs
2. Immediately apply PACC-directed therapy to kill the newly evolved PACCs
TARGETING PACCs OVERVIEW

- **Cell surface Antigens**
  - Ag-Ab conjugates
  - Stem cells

- **Stress Response**
  - GSH
  - Metals
  - NAC
  - Ca++

- **Protein Homeostasis**
  - Protein degradation
  - HSPs

- **Drug Resistance**
  - PgP

- **Metabolism**
  - Lipids
  - FAS

- **Autophagy**
  - Chloroquine
  - Metformin

- **Metformin**
  - AMPK

- **Senescence**
  - p53
  - Chk1
  - AurK A/B

- **Aneuploidy**
  - Centrosomes
  - KIFc1
  - Centrinone

- **Drug Resistance**
  - Experimental agents
  - Approved drugs

- **Stem cells**

- **Stress Response**

- **Protein Homeostasis**
  - bortezomib

- **Drug Resistance**

- **Metabolism**

- **Autophagy**

- **Mitotic Checkpoints**
  - p53
  - Chk1
  - AurK A/B
Our Super-PACC

Sarah Amend
Laurie Kostecka
Mikaela Mallin
Athen Olseen
Morgan Kuczler
Chi-Ju Kim
Kayla Myers
Liang Dong
Richard Zieren

Bob Austin
Bob Axelrod
Joel Brown
Emma
Hammarlund
Don Coffey

Yoon-Kyoung Cho
Phuoc Tran
Laura Buttitta
Anne Le
Sean Sun
Hui Zhang
Thomas Conrads
Claire Hur
Stavroula Sofou
James Hicks

NIH National Cancer Institute

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Congressionally Directed Medical Research Programs

Prostate Cancer Foundation
Curing Together.

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