Basics in cancer cell biology

• DNA repair

Ken A. Olaussen
OUTLINE

DNA damage response and DNA repair pathways
DNA repair and cancer
DNA repair biomarkers in breast cancer
DNA repair as anticancer target
DNA repair and targeted therapies
Perspectives and conclusions
Cells are continuously submitted to DNA damage

- High fidelity of DNA replication machinery: $10^{-10}$ spontaneous mutations/base
- Endogenous metabolic activities and environmental factors are continuously affecting DNA’s integrity (oxidative stress, alkylation agents, UV radiation ...)
- Lesions: chemical modification of DNA bases, abasic sites, base mismatches, chemical alkylation (adducts), intra- and inter-strand cross links, single- and double-strand breaks

<table>
<thead>
<tr>
<th>Endogenous DNA Damage</th>
<th>DNA Lesions Generated</th>
<th>Number Lesions/Cell/Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depurination</td>
<td>AP site</td>
<td>10000$^a$</td>
</tr>
<tr>
<td>Cytosine deamination</td>
<td>Base transition</td>
<td>100–500$^a$</td>
</tr>
<tr>
<td>SAM-induced methylation</td>
<td>3meA</td>
<td>600$^a$</td>
</tr>
<tr>
<td></td>
<td>7meG</td>
<td>4000$^a$</td>
</tr>
<tr>
<td></td>
<td>O$^6$meG</td>
<td>10–30$^b$</td>
</tr>
<tr>
<td>Oxidation</td>
<td>8oxoG</td>
<td>400–1500$^c$</td>
</tr>
</tbody>
</table>

Ciccia & Elledge, Molecular Cell, 2010
- Exogenous origins of DNA damage
- DSBs more dangerous and less frequent

<table>
<thead>
<tr>
<th>Exogenous DNA Damage</th>
<th>Dose Exposure (mSv)</th>
<th>DNA Lesions Generated</th>
<th>Lesions/day</th>
</tr>
</thead>
</table>
| Peak hr sunlight                      | —                   | Pyrimidine dimers, (6–4) photoproducts  | 100,000/day  
|                                       |                     |                                          |             |
| Cigarette smoke                       | —                   | aromatic DNA adducts                    | 45–1029      |
| Chest X-rays                          | 0.02^f,g,h           | DSBs                                    | 0.0008       |
| Dental X-rays                         | 0.005^f,g,h          | DSBs                                    | 0.0002       |
| Mammography                           | 0.4^f,g,h            | DSBs                                    | 0.016        |
| Body CT                               | 7^f                 | DSBs                                    | 0.28         |
| Head CT                               | 2^f,g               | DSBs                                    | 0.08         |
| Coronary angioplasty                  | 22^h                | DSBs                                    | 0.88         |
| Tumor PET scan (^18F)                 | 10^h                | DSBs                                    | 0.4          |
| ^131I treatment                       | 70–150^h            | DSBs                                    | 2.8–6        |
| External beam therapy                 | 1800–2000^j         | DSBs                                    | 72–80        |
| Airline travel                        | 0.005/hr^f          | DSBs                                    | 0.0002/hr    |
| Space mission (60 days)               | 50^k                | DSBs                                    | 2^i          |
| Chemobyl accident                     | 300^l               | DSBs                                    | 12^i         |
| Hiroshima and Nagasaki atomic bombs   | 5–4000^k            | DSBs                                    | 0.2–160^i    |

Ciccia & Elledge, Molecular Cell, 2010
BER pathway depends on the automodification (poly(ADP-ribosylation) (PAR) of PARP1 to release DNA from nucleosomes and recruit repair proteins.
Major DNA repair pathways and biomarkers

- **DNA lesion**
  - Single-strand break
  - Single-base damage
  - Bulky lesions
  - Crosslinks

- **Repair pathways**
  - BER
  - NER
  - TC-NER
  - O^6^MeG
  - Mismatch
  - Double-strand break

- **Proteins involved/biomarkers**
  - OGG1
  - PARP1
  - PARP2
  - DDB1/XPE
  - RNA pol β
  - CSA, CSB
  - XRCC1
  - ERCC1/XPF
  - PCNA
  - Pol δ
  - Pol ε
  - Pol β
  - PCNA
  - FEN 1
  - Ligase III
  - Ligase I
  - ERCC1

- **Other**
  - AGT
  - MSH2/MSH6
  - MLH1/PMS2
  - EXO1/PCNA/RCF
  - Pol δ
  - Pol ε
  - Pol μ
  - ATM
  - MRN complex
  - DNA PKs
  - Artemis
  - XRCC4-XLF
  - KU70, KU80
  - BRCA2/FANC
  - RAD51, FANC
  - PCNA

**Sources:**
- IMPAKT Breast Cancer Conference 2014
- Postel-Vinay, Nat Rev Clin Oncol 2012
DSB repair pathways
Alternative end-joining pathways

MMEJ (micro-SSA)

RAD52 or RAD59

LIG4/XRCC4

Ku70/Ku80

MRX

Alt-NHEJ

LIG1

Pol

Deletion

Annealing at pre-existing microhomologies

Annealing independent of pre-existing microhomologies

Decottignies, Front. Genet 2013
Numerous interconnexions between pathways
e.g. ERCC1 is implicated in several
« Mutability endow cancer cells with genetic alterations that drive tumor progression »

Hanahan and Weinberg, Cell 2011
Risk alleles in breast cancer: several DNA-damage response or repair-related genes: BRCA1/2, TP53, ATM, CHEK2…

Foulkes, NEJM 2008
“The balance of life” theory supports that DNA repair mechanisms can be an anticancer target 

DNA damage

Efficient DNA repair

Low DNA repair capacity

Genetic stability

Genetic instability

Inhibit DNA repair !!!

CELL SURVIVAL

Genetic divergence (cancer)

CELL DEATH

IMPAKT Breast Cancer Conference 2014
PARP inhibition is synthetic lethal in BRCA1-deficient tumors because HR pathway is a back-up pathway for unrepaired SSBs.
DNA Damage and Cellular Stress Responses

Targeting Abnormal DNA Repair in Therapy-Resistant Breast Cancers

Lisa A. Tobin¹, Carine Robert¹, Pratik Nagarla¹, Saranya Chumsri², William Twaddell³, Olga B. Ioffe³, George E. Greco², Angela H. Brodie³, Alan E. Tomkinson³, and Feyruz V. Rassool³

Altered expression of DNA repair proteins in breast cancer cell lines with acquired resistance to the antiestrogens tamoxifen and letrosole

The increase of Ligase 3 favors error-prone ALT NHEJ resulting in large deletions and chromosomal translocations in therapy-resistant cells

Tobin et al, Mol Cancer Res, 2012
Cell lines with acquired resistance to antiestrogen therapeutics are hypersensitive to a combination of DNA ligase 3 (L67) and PARP inhibitors. The hypersensitivity of the combination is followed by loss of efficient DSB-repair (although less error-prone).
ERCC1 deficient cell lines to explore synthetic lethality with candidate drugs/pathways
PARP is trapped onto the DNA by the PARP inhibitor leading to replication fork stalling and collapse.

PARP1/2 inhibitors selectively kills ERCC1-deficient cells

- PARP is trapped onto the DNA by the PARP inhibitor.
- Replication fork stalls due to the PARP inhibitor.
- ERCC1-deficient cells die due to replication fork collapse and collapse.
Cancer Research

ATR pathway inhibition is synthetically lethal in cancer cells with ERCC1 deficiency

Kareem N Mohri, Gina M Kavanaugh and David Cortez
Cancer Res. Published Online First March 24, 2014.

Breast cancer cell lines

![Graphs showing ATR pathway inhibition in breast cancer cell lines](image-url)
Nucleotide excision repair deficiency is intrinsic in sporadic stage I breast cancer

Jean J. Latimer, Jennifer M. Johnson, Crystal M. Kelly, Tiffany D. Miles, Kelly A. Beaudry-Rodgers, Nancy A. Lalanne, Victor G. Vogel, Amal Kanbour-Shakir, Joseph L. Kelley, Ronald R. Johnson, and Stephen G. Grant

aDepartment of Obstetrics, Gynecology and Reproductive Sciences, Magee–Womens Hospital, University of Pittsburgh Medical Center, Pittsburgh, PA 15213-3180; bHillman Cancer Center, University of Pittsburgh Cancer Institute, Pittsburgh, PA 15232-1301; cProgram in Biochemistry and Molecular Genetics, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261; dDepartment of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA 15261; Departments of eMedicine, fPathology, and gSurgery, Magee–Womens Hospital, University of Pittsburgh Medical Center, Pittsburgh, PA 15213-3180; and hDepartment of Environmental and Occupational Health, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA 15219-3130.
Remarkable loss of DNA repair proteins in TNBC compared to LABC

Table 3. Normalized and calibrated values (mean ± SD and median) of the different DNA repair genes in tumor samples.

<table>
<thead>
<tr>
<th>PATHWAY</th>
<th>GENE</th>
<th>TNBC (N=80)</th>
<th>LABC (N=70)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Median</td>
<td>Mean ± SD</td>
<td>Median</td>
</tr>
<tr>
<td>BER</td>
<td>PARP1</td>
<td>10.87±10.92</td>
<td>8.250</td>
<td>7.05±5.46</td>
</tr>
<tr>
<td>NER</td>
<td>ERCC1</td>
<td>0.71±0.66</td>
<td>0.633</td>
<td>6.87±20.77</td>
</tr>
<tr>
<td></td>
<td>XPA</td>
<td>0.10±0.08</td>
<td>0.080</td>
<td>0.16±0.21</td>
</tr>
<tr>
<td></td>
<td>XPF</td>
<td>23.35±55.61</td>
<td>5.108</td>
<td>68.93±107.05</td>
</tr>
<tr>
<td></td>
<td>XPG</td>
<td>0.66±0.71</td>
<td>0.468</td>
<td>1.51±3.52</td>
</tr>
<tr>
<td></td>
<td>XPD</td>
<td>0.276±0.360</td>
<td>0.186</td>
<td>6.68±10.26</td>
</tr>
<tr>
<td>FA</td>
<td>BRCA1</td>
<td>0.028±0.025</td>
<td>0.020</td>
<td>0.83±3.36</td>
</tr>
<tr>
<td></td>
<td>FANCA</td>
<td>0.138±0.279</td>
<td>0.057</td>
<td>0.27±0.71</td>
</tr>
<tr>
<td></td>
<td>FANCC</td>
<td>0.005±0.007</td>
<td>0.003</td>
<td>0.002±0.050</td>
</tr>
<tr>
<td></td>
<td>FANCD2</td>
<td>0.08±0.124</td>
<td>0.054</td>
<td>4.45±23.32</td>
</tr>
<tr>
<td></td>
<td>FANCF</td>
<td>0.026±0.027</td>
<td>0.018</td>
<td>0.451±1.170</td>
</tr>
<tr>
<td></td>
<td>PALB2</td>
<td>0.453±0.563</td>
<td>0.306</td>
<td>5.91±23.03</td>
</tr>
<tr>
<td>SENSOR</td>
<td>CHK1</td>
<td>0.179±0.300</td>
<td>0.108</td>
<td>11.51±46.44</td>
</tr>
</tbody>
</table>

FANCA mRNA expression in TNBC

Ribeiro E et al., PLoS ONE, 2013
Recent example: EGFR cooperates with glucose transporter SGLT1 to enable chromatin remodeling in response to ionizing radiation. Dittmann K, et al. Radiother Oncol. 2013
Inhibiting PI3K/AKT and Ras/ERK pathways

- Intracellular distribution of DNA repair proteins
- Transcription of DNA repair genes
- Phosphorylation of DNA repair proteins

EGFR

TKI

Internalization

DNA-PK

Ku70

Ku80

NHEJ

DSBs

Radiosensitivity

Baumann M et al., Radiother Oncol, 2007
Inhibiting PI3K/AKT and Ras/ERK pathways

- Intracellular distribution of DNA repair proteins
- Transcription of DNA repair genes
- Phosphorylation of DNA repair proteins

Clinical Cancer Research

Importance of EGFR/ERCC1 interaction following radiation-induced DNA damage

Gianmaria Liccardi, John A. Hartley and Daniel Hochhauser

*Clin Cancer Res* Published OnlineFirst April 29, 2014.

Baumann M et al., *Radiother Oncol*, 2007
Inhibiting PI3K/AKT and Ras/ERK pathways

- Intracellular distribution of DNA repair proteins
- Transcription of DNA repair genes
- Phosphorylation of DNA repair proteins

- Rad51/Rad50 expression
- HRR
- DSBs

Kryeziu K et al., *Mol cancer ther*, 2013
Inhibiting PI3K/AKT and Ras/ERK pathways

- Intracellular distribution of DNA repair proteins
- Transcription of DNA repair genes
- Phosphorylation of DNA repair proteins

- AKT and PI3K
- DNA-PKcs phosphorylation (NHEJ)
- ATM phosphorylation (NHEJ and HRR)
- ERK

IMPACT Breast Cancer Conference 2014

Meyn R et al., Radiother Oncol, 2009
PI3K inhibition increases indicators of DNA damage such as γ-H2AX, but decreases Rad51 foci and BRCA1 expression allowing synthetic lethality approaches (with PARPi) in HRR proficient cells.
Conclusive remarks

• DNA repair pathways important in cancer development and risk

• Complex interactions between DNA repair pathways give new therapeutic opportunities (synthetic letality)

• A more robust technology to select patients with DNA repair dysfunctionality is to be developed

• TKIs can change expression, intra-cellular distribution and activation of DNA repair proteins

• This indirect modulation inhibition of DNA repair might be combined with direct modulators (PARPi...)

• Better understanding of fundamental DNA repair mechanisms to be encouraged
Thank you!

Jean-Charles Soria
Fabrice André
Luc Friboulet
Sophie Postel-Vinay
Tony Sourisseau
Julien Adam
Florence Ponsonnailles
Angélique Robin
Nicolas Dorvault
Mei-Shiue Kuo-Cassin