Molecular Mechanisms of Therapy-Induced Cancer

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Mechanisms of therapy-induced transformation

EXPOSURE
(chemotherapy/radiotherapy)

CONSTITUTIONAL GENETICS

MODIFIERS
(smoking, diet, age etc)

MECHANISMS?
Somatic alterations

THERAPY-INDUCED CANCER
Chemotherapy-induced AML

ALKYLATING AGENTS (procarbazine, dacarbazine, temozolomide, cyclophosphamide, carmustine etc)
- Risk increases 1-2 yr, peaks 5-10 yr, then decreases
- Preceding myelodysplastic syndrome (4-7 hits)
- Chromosomal deletions or monosomies (Chr. 5/7)
- DNA mismatch repair defective with high mutation rate (after methylating agents)

TOPOISOMERASE INHIBITORS (etoposide, teniposide, doxorubicin, daunorubicin etc)
- Shorter induction period (median 2-3 yr) (1-2 hits)
- Balanced chromosomal translocations (MLL at 11q23) (MLL breaks map to topoisomerase binding sites)

Mechanisms of AML pathogenesis after methylating chemotherapy are well defined
Methylating agents select for DNA mismatch repair loss

azathioprine, 6-thioguanine, 6-mercaptopurine
Molecular mechanisms of transformation

DNA damage → Gene-specific effects → Altered phenotype

O6-methylguanine/6-thiopurine → Loss of DNA MMR (MLH1, MSH2, MSH6, PMS1, PMS2 etc) → Mutation acquisition/anti-apoptosis

DNA strand breaks at topoisomerase binding sites → MLL fusion gene → Dysregulated gene expression

IONISING RADIATION?
Radiation induced cellular damage

- Strand break induction
- Hydrolysis of water

Radiation exposure induces DNA double-strand breaks and DNA base – cytotoxic AND mutagenic –

Clustered DNA damage difficult to repair and can be converted to DNA DSBs.

DNA DSBs difficult to repair with high fidelity – translocations, loss (deletion) and gain (amplification) of genetic material.

How do we identify genetic lesions induced by radiation?

MODELS!
Radiogenic cancer in Hodgkin lymphoma survivors

- Risk of second cancer associated with radiation exposure
  - Positively correlated with dose
  - Inversely correlated with age
- Most common second cancers
  - Breast
  - Thyroid
- Contribution to risk of chemotherapy is unclear (site-specific?)
- Responsible for 20% of the mortality in HL survivors

*In vitro* model system based on the MCF10A breast epithelial cell line
47, XX, i(1)(q10), +del(1)(q12q32), der(3)t(3;9)(p14;p21), der(8)t(8;8)(q22;p23), der(9)t(3;9;5)(p14;p21;q23).

- reciprocal translocation between chromosomes 3 and 9 (deletion of CDKN2A/2B)
- non-reciprocal duplication of the end of the q arm of chromosome 5 to the derivative chromosome 9
- Isochromosome 1 and 8q duplication
SNP array karyotyping

Log2 ratio of 0 = 2 copies (diploid)

Chromosome 8
Immortality conferred by *CDKN2A* deletion

Chromosome 9 breakpoint

![Chromosome 9 breakpoint graph](image)

**COPY NUMBER:**

![Copy number plot](image)

Log2 Ratio

a

![CDKN2A marker](image)

Chromosome 3 breakpoint

![Chromosome 3 breakpoint graph](image)

**COPY NUMBER:**

![Copy number plot](image)

Log2 Ratio

b

![FAM19A1 marker](image)
Irradiation protocol

MCF-10 A

X-irradiation

Cell quiescence

Cell recovery

Cell Passage

Recovered cells

Cells cryopreserved

Cells for Re-irradiation

5Gy and 10Gy fractions to a cumulative dose of 80Gy +/- 10nM 17-β oestradiol
Radiogenic copy number alterations

Copy number alterations

>50kb discernible

- c-MYC has a pivotal function in growth control, differentiation and apoptosis.
- Over-expression has potent oncogenic activity.
- Dysregulation is a hallmark of many cancer types.
Evolution of the *c-MYC* locus in irradiated cells

Chromosome 8

**dup(8)(q12q24)**

predicted region of breakpoint

- Log2 ratio

*Evolution of the c-MYC locus in irradiated cells*
Evolution of the \textit{c-MYC} locus in irradiated cells

\textbf{Parental MCF-10A}

\textbf{80Gy Population}

\textbf{der(8)t(8;8)(q22;p23)}

\textbf{dup(8)(q12q24)}

\textbf{der(8)t(8;8)(q22;p23)}

\textbf{dup(8)(q12q24)}

\textbf{~59 Mb}
Evolution of the \textit{c-MYC} locus in irradiated cells

Chromosome 8
\textit{c-MYC}

Radiation-induced
c-MYC is prone to alteration in irradiated cells

- Translocation
  - der(8)t(8;8)(q22;p23)

- Duplication
  - der(8)t(8;8)(q22;p23)

- Triplication
  - der(8)t(8;8)(q22;p23)

Rare events – not part of the major clone
Radiation selects for c-MYC amplified cells

3 c-MYC copies (MCF-10A)

4 c-MYC copies (MCF-10A)

>4 c-MYC copies (MCF-10A)

Amplification correlated with expression

c-MYC (67 kDa)

GAPDH (37 kDa)

MCF-10A Cell population

Amplified cells are radioresistant

Multiple copies (<10) of c-MYC

Amplification correlated with expression

p=0.0027, two-way ANOVA

Colony efficiency (%)
Breast cancer (BC) in HL survivors

- Breast cancer after radiotherapy for Hodgkin lymphoma
  - N=18
  - Mean age at BC diagnosis 37 years, range 28-47 years
  - Mean age at HL diagnosis 21 years, range 16-29
  - Mean latency between HL and BC 15 years, range 7-24 years
  - Mean cumulative RT dose 19.9 Gy, range 1.2-42.7

- Breast cancer without a radiation aetiology
  - N=33
  - Mean age at BC diagnosis 40 years, range 32-49 years.
c-MYC amplification in BC

Mean c-MYC copy number

- **Sporadic**
- **Radiogenic**

Breast cancer cohort

Mean c-MYC/centromere 8 ratio

- **Sporadic**
- **Radiogenic**

Breast cancer cohort

- $p = 0.027$
- $p = 0.016$
- $p = 0.010$
Heterogeneous \textit{c-MYC} amplification

\textbf{a} SPO2

\begin{center}
\begin{tabular}{cc}
\textbf{c-MYC} & 3:2 \\
CEP8 & \end{tabular}
\end{center}

\begin{center}
\begin{tabular}{cccccccccccc}
& 1 & 2 & 3 & 4 & 5 & 6 & 7 & 8 & 9 & 10 & 11 & 12 & 13 & 14 \\
\hline
SPO2 & 5 & 53 & 8 & 6 & 21 & 5 & 2 & & & & & & & \\
& 38 & 62 & & & & & & & & & & & & & \\
\end{tabular}
\end{center}

\textbf{d} RAD10

\begin{center}
\begin{tabular}{cc}
\textbf{c-MYC} & 7:2 \\
CEP8 & \end{tabular}
\end{center}

\begin{center}
\begin{tabular}{cccccccccccc}
& 1 & 2 & 3 & 4 & 5 & 6 & 7 & 8 & 9 & 10 & 11 & 12 & 13 & 14 \\
\hline
RAD10 & 3 & 5 & 1 & 8 & 13 & 6 & 5 & 5 & & & & & & 1 \\
& 11 & 74 & 9 & 5 & 1 & & & & & & & & & & \\
\end{tabular}
\end{center}
Radiogenic cancer has high c-MYC expression

RAD13
Histoscore 0

RAD9
Histoscore 70.4

RAD6
Histoscore 158.8

Does radiation induce c-MYC amplification in non-immortalised primary cells?
Primary human mammary epithelial cells

PRIMARY Human Mammary Epithelial Cells (HuMEC)

X-irradiation 2Gy

Cell recovery

X-irradiation 1Gy

Cell senescence

Total dose 3Gy

X-irradiation 2Gy

Cell senescence

Total dose 4Gy
Primary human mammary epithelial cells

HuMEC FISH

Non-amplified Diploid
- c-MYC x2
- CEP8 x2
- IGH x2

Tetraploid
- c-MYC x4
- CEP8 x4
- IGH x4

Diploid Amplified
- c-MYC x7
- CEP8 x2
- IGH x2

Polyploid Amplified
- c-MYC Amp
- CEP8 x3
- IGH x4

Graph showing the percentage of nuclei in different ploidy states and c-MYC amplification levels after radiation exposure.
Elevated c-MYC expression drives cell proliferation and replicative stress. c-MYC amplification results in radiogenic angiosarcoma of the breast and radiogenic adenocarcinoma of the breast.

- **No c-MYC amplification** leads to radiation-induced atypical vascular lesion (non-malignant).
- **c-MYC amplification** post-radiation leads to attenuated PRDM1 (BLIMP1) expression, with OR Het 3.20 [0.89–11.52] and OR Hom 11.4 [3.23–40.25].
Molecular mechanisms of transformation

**DNA damage** → **Gene-specific effects** → **Altered phenotype**

- **O\(^6\)-methylguanine/6-thiopurine**
  - Loss of DNA MMR (MLH1, MSH2, MSH6, PMS1, PMS2 etc)
  → **Mutation acquisition**

- **DNA strand breaks** → **MLL fusion gene** → **Dysregulated gene expression**

**IONISING RADIATION?**

- **Base damage/strand breaks (clustered?)** → **c-MYC amplification** → **Transcriptional reprogramming**
Risk modifiers?

Critical to minimise healthy tissue exposure

Identify modifiers that change the relationship between mutation and cell death
Chemotherapy-induced mutation

**TK locus**
- MNU
- Ara-C

**HPRT locus**
- MNU
- Ara-C

WT

MSH2 deficient

**Survival Fraction (%)**
- MNU (mM)

**Cytotoxicity (%)**
- Mf (x10^-6)
Loss of MSH2 increases the mutagenic window

Identifying radiation risk modifiers?
- Secondary particles, bystander effects (likely mutagenic, are they also cytotoxic?)
- Proliferative index of the exposed healthy tissue (dividing cells fix more DNA to mutation)
- Volume of healthy tissue exposed (probability of SMN = mutations per cell X no. cells)
- Constitutional genetics (e.g. \textit{PRDM1} MAF 0.22; \textit{RB1} MAF \(10^{-5}\), \textit{TP53} rare)
- Acquired somatic alterations (DNA repair loss or apoptotic signalling dysfunction)
- Dose fractionation