Radiation-Induced Neoplastic Transformation *In Vitro*, Hormesis and Risk Assessment

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Neoplastic Transformation *In Vitro*

- Clear cancer-related endpoint (conversion of cells from a non-tumorigenic to a tumorigenic phenotype)
- Has proven to clearly describe high dose radiation carcinogenic effects *in vivo* (dose, dose-rate, LET, chemical promoters, chemical protectors)
- Is proving useful to explore possible mechanisms underlying the shape of the dose-response curve at low doses
Neoplastic Transformation *In Vitro*

- Limited in that the complexity of tissue microenvironment, immunosurveillance etc. is absent
- Limited in terms of difficulty in transforming primary cells, particularly primary human cells
- Limited number of cell systems amenable to quantitative assay of radiation effects
Neoplastic Transformation *In Vitro*

Most, if not all, *in vitro* systems are examining the transformation from some preneoplastic state to the neoplastic state. Is this relevant?
Neoplastic Transformation *In Vitro*

- Most humans are carrying a burden of preneoplastic cells in their bodies.
- Given that they are part way down the path to cancer they could be considered a particularly important target.
- According to Folkman, most tissues harbor “dormant” tumorigenic cells. It is unclear whether in vitro studies can mimic any possible radiation “activation” of such cells.
Neoplastic Transformation *In Vitro*

- Amenable to the study of low dose effects, but requires extensive and labor-intensive experimentation to achieve even reasonable statistics.
- Two experimental systems (*C3H10T1/2* and the *HeLa x skin fibroblast human hybrid cell assay*) have demonstrated hormetic responses at low doses of low LET radiations and a *J*-shaped dose-response curve.
HeLa X Skin Fibroblast Hybrid Cells And
The Study of Tumor Suppressor Function

Tumorigenicity and IAP expression are negatively
regulated by a suppressor on chromosome 11

HeLa T/IAP+

X

Skin Fibroblast NT/IAP-

Hybrid Cell, CGL 1
NT/IAP-

Hybrid Cell
CGL3 & CGL4
T/IAP+
Schema Assay of HeLa X Fibroblast Human Hybrid Cell Neoplastic Transformation By Radiation

- Plate Cells
- Irradiated (late log/early plateau)
- 50 viable cells/cm²
- 2 days
- 24 h
- 21 days
- 5 viable cells/cm²
- 10 days
- Score Survivors
- Score Transformants
- 50 viable cells/cm²
- 24 h
Dose Dependence of Cs-137 γ Radiation Induced Neoplastic Transformation In Vitro.

A. Immediate Plating  95%CI

B. Delayed Plating  95%CI

Dose Dependence of Cs-137 $\gamma$ Radiation Induced Neoplastic Transformation In Vitro: Low Dose Deviation from Linear Extrapolation from High Doses.

A. Immediate Plating 95%CI

B. Delayed Plating 95%CI

Dose-response curve for neoplastic transformation of human hybrid cells by 60 kVp X-rays

Transformation Frequency ($\times 10^5$) vs. Dose (cGy)
Dose-response curve for neoplastic transformation of human hybrid cells by 28 kVp X-rays
Dose-response curve for neoplastic transformation of human hybrid cells by 28 kVp X-rays: Two data sets normalized to the same spontaneous frequency.

Neoplastic Transformation In Vitro

- In summary, we have routinely observed J-shaped dose-response curves following HDR treatment and are interested in mechanisms underlying this shape.
- Studies with the human hybrid cell system indicate that there may be at least two phenomena involved.
- Low dose hyper-radiosensitivity of a transformation-sensitive subpopulation (G2?).
- Induction of DNA repair. Future studies will examine the roles of NHEJ and HR using RNAi.
Neoplastic Transformation In Vitro

We have recently performed dose-rate studies using an I-125 irradiator that is allowing us to look at dose-rates <2.0 mGy/min of ca. 30 keV photons with the aim of comparing the data with that seen at high doses for high dose-rates of similar energy (28 kVp) mammography x-rays (Heyes and Mill, Rad. Res. 162:120, 2004; Ko et al., Rad. Res. 162:646, 2004).
I-125 IRRADIATOR

6mm Pb-lined lucite box
10 x 11 seed array
Initial activity = 1.0 mCi/seed
Initial dose rate = 2.2 mGy/min
T-75 Flask

0.5 cm forward scatter

2 cm backward scatter
Dose Linearity

$y = 12.08x$

$R^2 = 0.9944$
Dose-Response for Induction of Neoplastic Transformation at Low Dose Rates

![Graph showing dose-response relationship with transformation frequency and dose in mGy. The graph includes a line of best fit and data points with error bars. The slope of the line is labeled as 1.9 mGy/min.](image-url)
Dose-Response for Induction of Neoplastic Transformation at Low Dose Rates

Dose (mGy) vs. Transformation Frequency ($\times 10^5$)

- Data points for $0.91 \text{ mGy/min}$

Graph shows an increase in transformation frequency with increasing dose.
Dose-Response for Induction of Neoplastic Transformation at Low Dose Rates

Transformation Frequency (x10^5)

Dose mGy

0.47 mGy/min
Dose-Response for Induction of Neoplastic Transformation at Low Dose Rates

Transformation Frequency ($x10^5$) vs. Dose (mGy)

- 0.19 mGy/min
Dose-Response for Induction of Neoplastic Transformation at Low Dose Rates

- 1.9 mGy/min
- 0.91 mGy/min
- 0.47 mGy/min
- 0.19 mGy/min
Neoplastic Transformation In Vitro: Relative Risk vs. Dose and Dose-Rate
Neoplastic Transformation In Vitro: Relative Risk vs. Dose and Dose-Rate
Neoplastic Transformation In Vitro

- Not unexpectedly, the data show that as the dose-rate is decreased the effectiveness of the radiation in inducing neoplastic transformation decreases.

- At dose-rates of 0.19 and 0.47 mGy/min no induction of transformation is seen up to a dose of 1000 mGy, and the data suggest that there may be a suppression of transformation at these dose-rates, i.e. certainly do not conform to the LNT model.
Neoplastic Transformation *In Vitro*

- Now to the $64,000 question! Is there any evidence for J-shaped dose-response curves in the animal and human epidemiological data?
- The answer is a qualified yes, i.e. there are trends in this direction but these are not significant.
Several *in vivo* studies of the effects of low doses of low LET radiation cannot rule out the notion of a threshold dose.

Ullrich and Storer (1979) indicated thresholds of 0.22 Gy for myeloid leukemia, and 0.10 Gy for ovarian, pituitary and Harderian gland tumors, fit the data as well as the LNT model.
Figure XXVIII. Relative risk of leukaemia in survivors of the atomic bombings [L44]. The diagram on the right shows the low-dose region in detail.
Little and Muirhead (IJRB 74:47-180, 1998) indicated that the dose-response was curved but with “absence of evidence of threshold”, yet a:

- Fit of a threshold model with a linear dose response above the threshold resulted in a best estimate of a threshold of 0.16 Sv (95% CI 0.05-0.40) with two-sided p=0.001 for departure from a threshold of zero
- Fit of a threshold model with a quadratic term above the threshold resulted in a best estimate of a threshold of 0.09 Sv (95% CI <0.00-0.29) with two-sided p=0.07 for departure from threshold
Neoplastic Transformation In Vitro

- It is clear that the degree of suppression and threshold dose is going to be target tissue-dependent.
- It will also be very dose-rate dependent and there are very little human epidemiological data at low dose-rates.
- Animal data at low dose-rate often show apparent thresholds for low LET radiation.
Neoplastic Transformation In Vitro

Can in vitro experiments provide data that can give an idea of the relative risk of cancer induction in humans?

For a whole lot of reasons alluded to earlier one would say no. However, it is interesting that when compares relative risks from epidemiologic data with those from in vitro transformation data there is surprising agreement, at least for certain tumors.
Relative Risk vs. Dose

Comparison of In Vitro and Epidemiologic Data

- Cs-137
- 60 kVp
- Co-60
- Leukemia-mortality
- Leukemia-incidence
- Breast-incidence
- Breast-mortality

Dose (cSv)

Relative Risk
Neoplastic Transformation In Vitro

- What about a comparison of risk estimations (epi vs. in vitro) for low dose-rate radiation?
- Very little epi data for chronic exposures, but there is a study from Sweden (Lundell et al., Rad. Res. 151:626-632, 1999) for breast cancer as a result of low dose-rate (median dose rate to breast of 0.4 mGy/min) radium plaque treatments for hemangiomas.
- Data from this study is included in a paper on radiation-induced breast cancer by Preston et al., Rad. Res. 158:220-235, 2002).
- Both of these papers concluded that the data were consistent with the LNT model BUT Lundell did indicate that only at doses >1 Gy was there a positive association with breast cancer risk.
Relative Risk vs. Dose
Breast cancer following LDR treatment for hemangioma

incidence rate
breast cancer hemangioma
Gothenburg & Stockholm

G. Stanford
Breast Cancer Incidence Rate vs. Dose
Breast cancer following LDR treatment for hemangioma

breast cancer, hemangioma Gothenburg & Stockholm

Radiation effects on breast cancer risk: a pooled analysis of eight cohorts.
Relative Risk vs. Dose for Breast Cancer
Comparison of HDR and LDR Epidemiologic Data

![Graph showing Relative Risk vs. Dose](image)

Comparison of Relative Risk for Transformation with Breast Cancer Induction at High and Low Dose-Rates

- LDR-BCa (Rad. Res. 158:220, 2002) 0.16-0.83 mGy/min
- LDR (Cell Transformation Data - 2005) 0.47 mGy/min
- HDR (Cell Transformation Data - 2001) >3 mGy/min
Neoplastic Transformation *In Vitro*, Hormesis and Risk Assessment

- In conclusion, neoplastic transformation *in vitro* has demonstrated strong evidence for hormetic effects of low doses of low-LET radiation at both high and low dose-rates.
- Neoplastic transformation *in vitro* has proven capable of making relative risk estimates of cancer incidence that compare well with those seen epidemiologically for breast cancer and leukemia.
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### Dose Distribution

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<th>Position #</th>
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<th>Calculated</th>
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<td>200 cGy</td>
</tr>
<tr>
<td>2</td>
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<td>80 cGy</td>
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<tr>
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<td>50 cGy</td>
<td>50 cGy</td>
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<tr>
<td>4</td>
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<td>20 cGy</td>
</tr>
<tr>
<td>5</td>
<td>100 cGy</td>
<td>100 cGy</td>
</tr>
</tbody>
</table>

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![Dose Distribution Graph](image)

- **200 cGy**: Measured and Calculated
- **80 cGy**: Measured and Calculated
- **50 cGy**: Measured and Calculated
- **20 cGy**: Measured and Calculated
- **100 cGy**: Measured and Calculated

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For a detailed analysis of dose distribution, please refer to the PDF document.
Dose-Response for Induction of Neoplastic Transformation at Low Dose Rates
Breast Cancer Incidence Rate vs. Dose
Breast cancer following LDR treatment for hemangioma

"It was the contribution of subjects with breast doses > 1.0 Gy that produced a positive association between dose and the subsequent breast cancer risk."

Radiation effects on breast cancer risk: a pooled analysis of eight cohorts.
Relative Risk vs. Dose
Breast cancer following LDR treatment for hemangioma

Radiation effects on breast cancer risk: a pooled analysis of eight cohorts.

-breast cancer
- incidence rate
-hemangioma Gothenburg & Stockholm

RR, risk relative to first dose interval
organ dose / mGy
Relative Risk vs. Dose for Breast Cancer
Comparison of HDR and LDR Epidemiologic Data

![Graph showing relative risk vs. dose for breast cancer with data from LDR-BCa and HDR-BCa](image-url)
Relative Risk vs. Dose for Breast Cancer
Comparison of HDR and LDR Epidemiologic Data

![Graph showing relative risk vs. dose for breast cancer.]

The shape of the dose response curve for radiation-induction of cancer by low LET radiation - Two official positions

- BEIR VII Phase 2 report from the U.S. National Academy of Sciences supports the LNT model down to zero dose.
- The French Academy of Sciences and National Academy of Medicine does not support the LNT model at doses <100 mSv.
- The French committee was of the opinion that the evidence from laboratory, and some epidemiology studies, was sufficiently strong to indicate that the LNT model will likely overestimate risk at doses <100 mSv, and almost certainly at doses <10 mSv.
In conclusion, neoplastic transformation in vitro has been demonstrated to describe dose-response curve shapes that are consistent with those seen in epidemiologic studies of radiation-induced breast cancer and leukemia.