Dual Nucleosomal Double Strand Breaks are the Key Effectors of Curative Radiation Therapy: Supplement 2, 3

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TP53: Damage Sensors & Modification Sites & Associated Upstream Proteins

Close Up of TP53 the "Guardian of the Genome" The Cellular "Brain" Behind: DNA Repair, Cell Cycling Survival, Senescence & Apoptosis also Indicating Key Up- and Down- Stream TP53 Associated Pathways Such as Damage Sensors and DNA Repair Effectors!

DNA Damage Initial Response:

Phosphorylation (P)
Acetylation (A)
Methylation (M1,2,3)
PARylation (PA) ADPribosylation (A) Glycosylation (G)
Carboxyl Methylation (M) Isomerisation (I) Crotonylation (C)
Asparagine Methylation (M) β-Hydroxybutyrylation (β) Nitration (N)

Damage Recovery:

Ubiquitylation
Ufmylation
Fatylation
Isgylation
Glutathionylation (G) Sumoylation
Hydroxylation (H) Neddylation
Prostaglandin conjugation (P)

Amino Terminal — TAD1 TAD2 PRR NRD N A - Binding Domain NLS T D CTD — Carboxyl Terminal

Start up of Effective DNA Repair by NHEJ & HR Require 1/2 Gy to Phosphorylate Both ATM & CHK2 and NHEJ then p53 at Serine 15 & 20! Causing Low Dose Hyper Sensitivity and Low Dose Apoptosis in Most Normal Tissues!

NHEJ: Non Homologous End Joining
MHEJ: Micro Homologous End Joining
TMEJ: Pollymeras θ Mediated End Joining
HDR: Homology Directed Repair
IHR: Inter Homolog Recombination
HR: Homologous Recombination

Apoptosis
DSB DNA Repair
Cell Cycle Block
Senescence
Senescence

Low Dose Apoptosis
ln(S)
1
0.5
0.1
0 1/2 Gy 1 2 3 4 5 D/Gy

Late, Slow Responding Tumor. Long Cell Cycle, Ample Time for Repair. Big Low Dose Shoulder, High Dose Sensitive (eg Brain mut TP53)
Acute, Fast Responding Tumor, Short Cell Cycle Little Time for Repair Small Shoulder, High Dose Resistant (eg Kidney mutant TP53)
Intact, Low Dose Hyper Sensitive Normal Tissues (eg. Lung Epithelia wt TP53)
e-aD All Unhit and Undamaged Cells
≈bDe-cD All Sublethal Repairable Damage
wt TP53 pSer 15+20
Lithium to Carbon Ion Bragg Peak Responses (≈ Independent of TP53 & Tumor or Normal Tissue!)
RHR
LQ
LQ

ATM 1/4Gy
CHK2 1/2 Gy Ser20 18 DSB! HR
DYRK2 ATM p38K ≈2GyE Ser46
NHEJ 9DSB! Ser15
p53

Mild Cellular Stress
Mild DNA Damage
σ_n Non-Homologous Repairable Damage
σ_h Homologically Repairable Damage "RHR"
Severe Cellular Stress
Severe DNA Damage
σ_i Un Repairable Injury

Ser20 Ser15 p53 Ser46 p53DINP1 Ser46-kinases
>1/2 Gy
Growth Arrest
p21
p53R2
GADD45
DNA Repair
P53RFP
Survival: Continued Cell Cycling
Senescence
Autophagy
High Dose Apoptosis
Ceramide
NHEJ &/or HR Misrepair
≈2 DDSB! ≈2 GyE
p53AIP1
Caspase 3!
Apoptosis

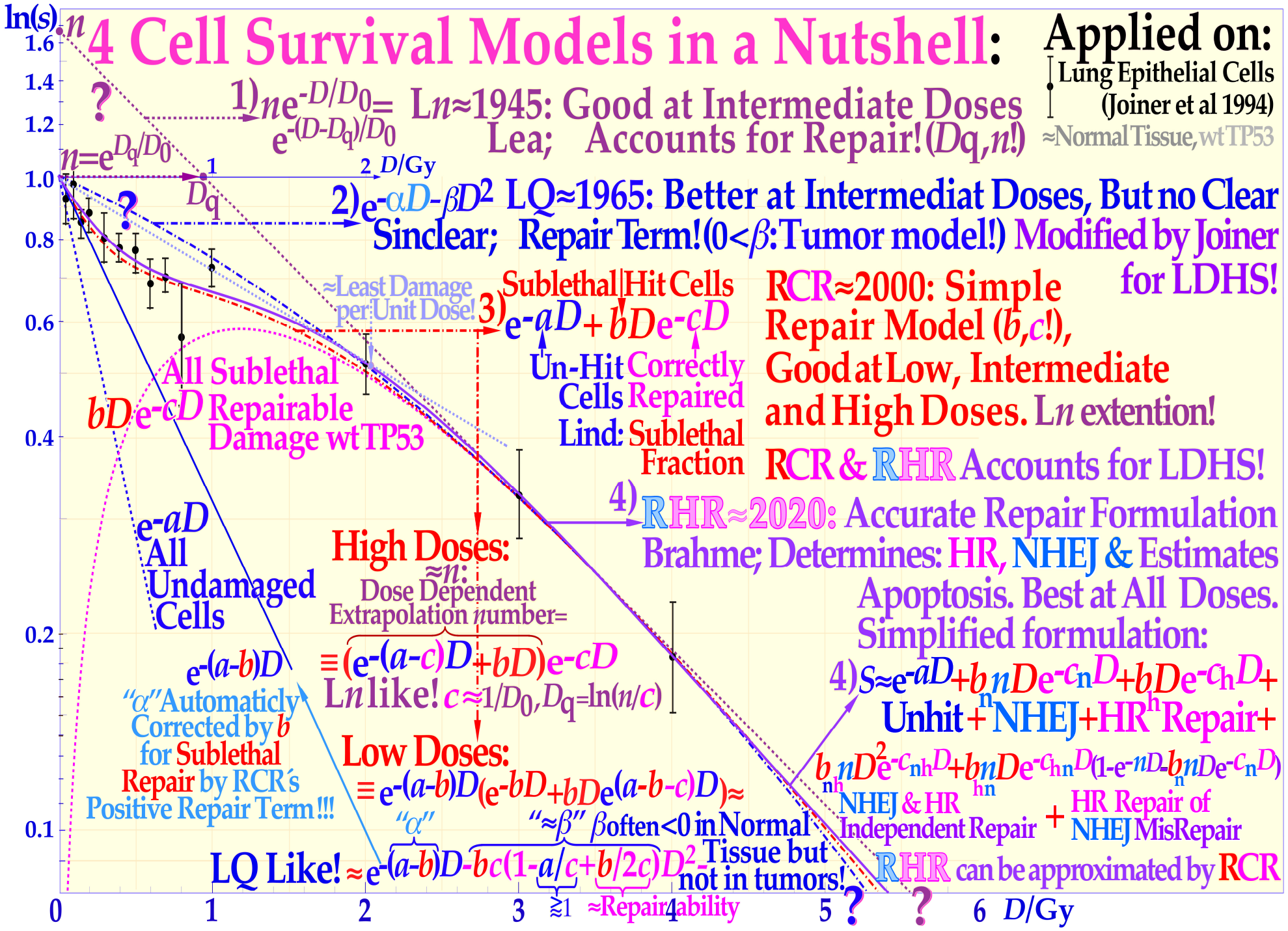
Nucleosomal DNA
Nucleosome Histone Octamer Core
"δ-electron"
δ-e⁻ Track End ≈1MGy
→DDSB!

Facilitates "Molecular Radiation Therapy" Using Lithium-Boron Ions with Mainly a Low LET in Normal Tissues! Preserves the Fractionation Window a Steep Dose Response and High P_+!
Lithium-Boron Ions!

Low LET: < 10 eV/nm Induces DNA Repair and Cell Survival! (Except at: Bragg Peak)
Total Dose
<10 eV/nm
10-20 eV/nm
<10eV/nm NHEJ
HR
NHEJ
σ_n σ_h σ_n

High LET Bragg Peak 700 eV e⁻
≈3-5mm
20<LET<250eV/nm
With Increased Tumor Cell Pacification & Inactivation by Senescence, Autophagy & Apoptosis!

Supplement 2. The known detailed response of the TP53 gene product shows the complexity of some of the underlying processes that largely determine the cellular response to radiation. Common p53 mutation sites are indicated by fine blue dashed lines mainly in the DNA binding domain. This figure shows some of the inner workings of p53 in its rather complex downstream pathways (cf [40]: Figure 1 for further downstream pathway details) the lower half for the cellular response to radiation and cell survival, Simplified view of how p53 reacts to mild and severe genetic stress (the lower half). Mild stress phosphorylates the serine 15 and 20 sites on p53 by ATM and CHK2, resulting in cell cycle block and effective DNA repair after 18 DSBs or 1⁄2 Gy. This results in LDHS and low-dose apoptosis (LDA) in normal tissues. Local high doses or high ionization densities are result in DDSBs that increase the severity of the damage. This results in phosphorylation of the serine 46 site, e.g., via ATM and/or p38K, and a high dose apoptotic (HDA) response may be triggered. Most tumors that often have a mutant TP53 gene, as seen in the lower left cell survival insert, have often lost both LDA, LDHS and HDA. Therefore, lithium ions (lower part) will allow unique therapeutic use by inducing a massive apoptotic-senescent tumor cell response mainly within the Bragg peak region (σ_h homologically repairable damage and σ_i direct inactivation cross-sections, see sections 5 and 6), but in front of and beyond the Bragg peak, the *LET* is low and mainly induces non-homological easily and rapidly repairable damage (σ_n cross-sections [1, 5-9, 41-43]). Thus, the low *LET* and dose fractionation window is fully retained (Figure 4 [27]). Common p53 mutation sites are indicated by fine blue dashed lines mainly in the DNA binding domain.


ln(s)
n
4 Cell Survival Models in a Nutshell:
Applied on:
Lung Epithelial Cells
(Joiner et al 1994)
≈Normal Tissue, wt TP53
1) $ne^{-D/D_0}=e^{-(D-D_q)/D_0}$ Ln≈1945: Good at Intermediate Doses
Lea; Accounts for Repair! (Dq,n!)
$n=e^{D_q/D_0}$
D_q
1
2 D/Gy
2) $e^{-\alpha D-\beta D^2}$ LQ≈1965: Better at Intermediat Doses, But no Clear
Sinclear; Repair Term! (0<β: Tumor model!) Modified by Joiner for LDHS!
≈Least Damage per Unit Dose!
3) $e^{-aD}+bDe^{-cD}$ Sublethal|Hit Cells
Un-Hit Correctly Cells Repaired
Lind: Sublethal Fraction
RCR≈2000: Simple Repair Model (b,c!), Good at Low, Intermediate and High Doses. Ln extention!
All Sublethal
bDe^{-cD} Repairable Damage wt TP53
RCR & RHR Accounts for LDHS!
4) RHR≈2020: Accurate Repair Formulation
Brahme; Determines: HR, NHEJ & Estimates Apoptosis. Best at All Doses.
Simplified formulation:
e^{-aD} All Undamaged Cells
High Doses: ≈n: Dose Dependent Extrapolation number=
$\equiv(e^{-(a-c)D}+bD)e^{-cD}$
Ln like! $c\approx 1/D_0, D_q=\ln(n/c)$
$e^{-(a-b)D}$
"α" Automaticly Corrected by b for Sublethal Repair by RCR's Positive Repair Term!!!
Low Doses:
$\equiv e^{-(a-b)D}(e^{-bD}+bDe^{(a-b-c)D})\approx$
"α" "≈β" β often<0 in Normal Tissue but not in tumors!
LQ Like! $\approx e^{-(a-b)D-bc(1-a/c+b/2c)D^2}$
≈Repairability
4) $S\approx e^{-aD}+b_n nDe^{-c_n D}+bDe^{-c_h D}+$
Unhit + ^{n}NEJ + HR^{h} Repair +
$b_{nh}nD^2e^{-c_{nh}D}+b_{hn}nDe^{-c_{hn}D}(1-e^{-nD-b_{nh}nDe^{-c_n D}})$
NHEJ & HR Independent Repair + HR Repair of NHEJ MisRepair
RHR can be approximated by RCR
0
1
2
3
4
5
6
D/Gy
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.1

Supplement 3. The development of the description of the shape of the cell survival curve during the last ≈ hundred years from the linear exponential model with a back extrapolated effective initial cell number (n, Ln) and todays dominating linear quadratic formula (LQ) that does not even account separately for cell repair as Ln does. The more recent Repairable Conditionally Repairable model handles the cellular repair much better (cf supplement 3 for further details) and separates it from unhit survival, whereas the most recent Repairable Homologous Repairable (RHR) formulation further accounts separately for non-homologous and homologous recombination repair as shown in the lower right corner, and can estimate the apoptotic fraction and the individual repair processes for further details: [1, 9, 17, 27]. At high doses the RCR expression can be seen as an extension of the Ln model whereas at low doses a resemblance to the LQ model is seen but generally with a negative β value due to LDA mainly missing in often TP53 mutant tumors. Interestingly, the LDHS caused by LDA initiate a rather radiation resistant cell survival phase towards 2 Gy where minimal damage is induced in normal tissues per unit dose to an underlying target volume! Interestingly, during the 125 years of curative radiation therapy, we have already found how to fractionate radiation treatments to maximize curability using the well established 2 Gy/Fr dose regiment. This we can understand now to be due to the fact that the least damage per unit dose is obtained between 1.8 and 2.3 Gy/Fr, as indicated by the fine dotted pail blue tangent line with the shallowest slope possible through a point on the curve and the point of unit survival as seen in the Figure [2]. This generates a Fractionation Window in LDHS normal tissues, indicating that the maximum dose to organs at risk should be ≤2.3 Gy/Fr, and by necessity of a low *LET* !! For further details see [1, 9, 17, 27, 41, 44, 59]. The existence of a "fractionation window" where radiation therapy works well was established in the era of parallel-opposed beams with almost equal doses to the tumor and organs at risk [17]. Now as we understand the underlying molecular mechanisms as seen in Figure 3a and Supplement 2 we know how it should be used also in the present era where we use biologically optimized Intensity Modulation Radiation Therapy (IMRT) to maximize the complication-free cure. Normal tissues at risk should still be ≤2.3 Gy/Fr, but most conservative oncologists keep using the old usual well established 2 Gy/Fr also with IMRT, which is suboptimal, almost 10 Gy lower total doses are possible with the most recent new approaches using the presently introduced more accurate radiation biology [17].