Dual Nucleosomal Double Strand Breaks are the Key Effectors of Curative Radiation Therapy: Supplement 1

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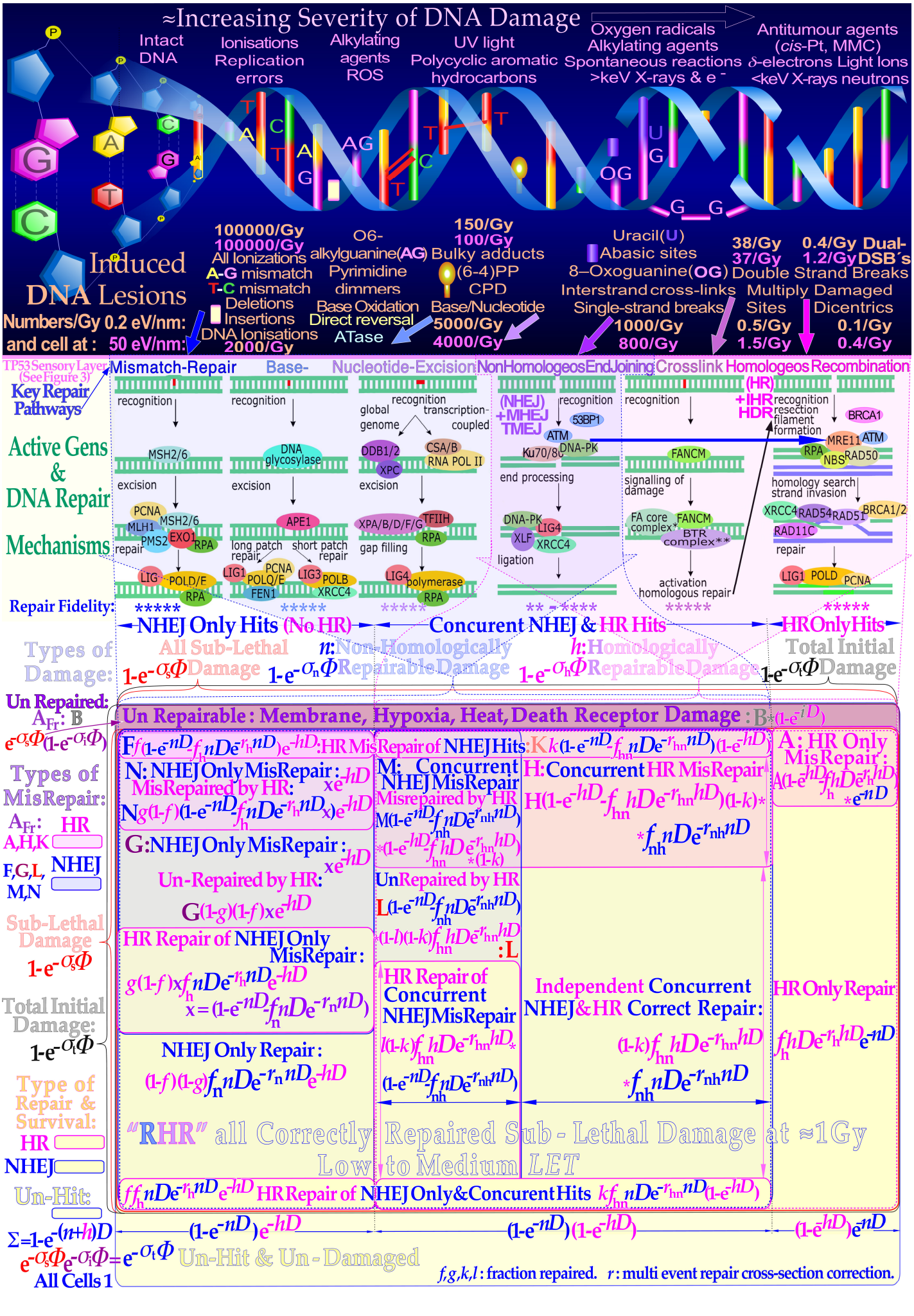
≈Increasing Severity of DNA Damage
Intact DNA
Ionisations Replication errors
Alkylating agents ROS
UV light Polycyclic aromatic hydrocarbons
Oxygen radicals Alkylating agents Spontaneous reactions >keV X-rays & e⁻
Antitumour agents (cis-Pt, MMC) δ-electrons Light Ions <keV X-rays neutrons
Induced DNA Lesions
Numbers/Gy 0.2 eV/nm: and cell at : 50 eV/nm:
100000/Gy 100000/Gy All Ionizations
A-G mismatch
T-C mismatch
Deletions
Insertions
DNA Ionisations 2000/Gy
O6-alkylguanine(AG)
Pyrimidine dimmers
Base Oxidation
Direct reversal
ATase
150/Gy 100/Gy Bulky adducts
(6-4)PP
CPD
Base/Nucleotide 5000/Gy 4000/Gy
Uracil(U)
Abasic sites
8–Oxoguanine(OG)
Interstrand cross-links
Single-strand breaks
1000/Gy 800/Gy
38/Gy 37/Gy Double Strand Breaks
Multiply Damaged Sites 0.5/Gy 1.5/Gy
0.4/Gy Dual-1.2/Gy DSB´s
Dicentrics 0.1/Gy 0.4/Gy
TP53 Sensory Layer (See Figure 3)
Key Repair Pathways
Active Gens & DNA Repair Mechanisms
Mismatch-Repair
Base-
Nucleotide-Excision
NonHomologeos EndJoining (NHEJ) +MHEJ TMEJ
Crosslink
Homologeos Recombination (HR) +IHR HDR
recognition
MSH2/6
excision
PCNA MLH1 MSH2/6 PMS2 EXO1 RPA repair
LIG POLD/E RPA
DNA glycosylase
APE1
long patch repair
short patch repair
LIG1 PCNA POLQ/E FEN1
LIG3 POLB XRCC4
global genome
transcription-coupled
DDB1/2 XPC
CSA/B RNA POL II
XPA/B/D/F/G TFIIH RPA
gap filling
LIG4 polymerase RPA
53BP1
ATM
Ku70/80 DNA-PK
end processing
DNA-PK LIG4 XLF XRCC4
ligation
FANCM
signalling of damage
FA core complex* FANCM BTR complex**
activation homologous repair
resection filament formation
BRCA1
MRE11 ATM RPA NBS RAD50
homology search strand invasion
XRCC4 RAD54 RAD51 BRCA1/2 RAD11C
repair
LIG1 POLD PCNA
Repair Fidelity:
*****
** - *****
NHEJ Only Hits (No HR)
Concurent NHEJ & HR Hits
HR Only Hits
Types of Damage:
All Sub-Lethal Damage
1-e^-σsΦ
n:Non-Homologically Repairable Damage
1-e^-σnΦ
h:Homologically Repairable Damage
1-e^-σhΦ
Total Initial Damage
1-e^-σtΦ
Un Repaired: A_Fr: B
e^-σsΦ(1-e^-σtΦ)
Un Repairable : Membrane, Hypoxia, Heat, Death Receptor Damage : B*(1-e^-iD)
Types of MisRepair:
A_Fr: HR
A,H,K
F,G,L, M,N NHEJ
Sub-Lethal Damage 1-e^-σsΦ
Total Initial Damage: 1-e^-σtΦ
Type of Repair & Survival:
HR
NHEJ
Un-Hit:
Σ=1-e^-(n+h)D
e^-σsΦe^-σtΦ=e^-σtΦ
All Cells 1
F f(1-e^-nD-f_h nD e^-r_h nD)e^-hD :HR MisRepair of NHEJ Hits
K k(1-e^-nD-f_hn nD e^-r_hn nD)(1-e^-hD)
N: NHEJ Only MisRepair : xe^-hD
MisRepaired by HR: Ng(1-f)(1-e^-nD-f_h nD e^-r_h nD x)e^-hD
M: Concurrent NHEJ MisRepair Misrepaired by HR
M(1-e^-nD-f_nh nD e^-r_nh nD)*(1-e^-hD-f_hn hD e^-r_hn hD*(1-k))
H:Concurrent HR MisRepair
H(1-e^-hD-f_hn hD e^-r_hn hD)(1-k)*f_nh nD e^-r_nh nD
A: HR Only MisRepair: A(1-e^-hD-f_h hD e^-r_h hD)*e^-nD
G:NHEJ Only MisRepair : xe^-hD
Un-Repaired by HR: G(1-g)(1-f)xe^-hD
UnRepaired by HR L(1-e^-nD-f_nh nD e^-r_nh nD)*(1-l)(1-k)f_hn hD e^-r_hn hD :L
HR Repair of NHEJ Only MisRepair : g(1-f)x f_h nD e^-r_h nD e^-hD
x=(1-e^-nD-f_n nD e^-r_n nD)
HR Repair of Concurent NHEJ MisRepair l(1-k)f_hn hD e^-r_hn hD*(1-e^-nD-f_nh nD e^-r_nh nD)
Independent Concurrent NHEJ&HR Correct Repair: (1-k)f_hn hD e^-r_hn hD *f_nh nD e^-r_nh nD
HR Only Repair f_h hD e^-r_h hD e^-nD
NHEJ Only Repair : (1-f)(1-g)f_n nD e^-r_n nD e^-hD
"RHR" all Correctly Repaired Sub-Lethal Damage at ≈1Gy Low to Medium LET
f f_h nD e^-r_h nD e^-hD HR Repair of NHEJ Only&Concurent Hits k f_hn nD e^-r_hn nD(1-e^-hD)
(1-e^-nD)e^-hD
(1-e^-nD)(1-e^-hD)
(1-e^-hD)e^-nD
Un-Hit & Un-Damaged
f,g,k,l : fraction repaired. r : multi event repair cross-section correction.

Supplement 1. Different parts of the DNA damage spectrum induce a number of associated repair pathways that determine the possibility of the cell repairing its DNA and recovering from the damage. The current theory, for simplicity, split the damage and repair into two main groups, depending on whether it was mild and easily and rapidly repaired or more complex, requiring the application of the slower high fidelity Homologous Recombination (HR) machinery. This HR contribution is needed to clear possible misrepared DSBs likely to be produced by nonhomologous end joining (NHEJ) at local high doses or *LET*s. The non-homologically repairable damage includes plain NHEJ and all the mechanisms to the left of it in the lower half of the figure. The TP53 sensory layer between damage and repair is shown in more detail in Figure 3a describing the wide range of sensory proteins and cellular response mechanisms (Figure 2a developed from [1, 9, 33, 34]). The speed of the Ku-DNApk heterodimer complex and TP53 recruitment to a DNA DSB, a few seconds, makes it the most likely starting point of all DSBs absolutely necessary not to lose the right order in which the DNA ends belong together (horizontal blue arrow NHEJ->HR "switch" when p53 senses repair problems as in Figure 10 [1, 4, 9, 17, 27]). There are also a number of other connections between NHEJ and HR as indicated previously such as that HR assists if a key NHEJ repair gene is knocked out and vice versa and HR may clear some NHEJ misrepair [1, 9, 27]. The two main groups of DNA damage: non-homologically-repairable damage (***n***) and the group requiring the homologues recombination (HR) machinery (***h***). A single cell can have one or the other or both ***n***- and ***h***-type damage resulting in different probabilities of repair (yellow) and misrepair (upper shaded areas, cf Eq 1-9). Interestingly, the new repair formulation is consistent with a fair probability of HR repair of NHEJ only and concurrent misrepair. The capital A-N are the fraction of each misrepair process that may lead to apoptosis; see [1, 17, 27] for further details.