Forty years of the L5178Y model: in pursuit of factors that determine the cellular sensitivity to DNA damaging agents

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L5178Y-S (LY-S) subline is the longest known mammalian radiation sensitive cell line; it was isolated from the parental L5178Y line (later called LY-R) and characterised by Alexander and Mikulski in 1961 [1]. Professor Peter Alexander gave the cells to Dr. Janusz Beer who took them to his Warsaw laboratory. So, the cells became a favourite object of studies at the Department of Radiobiology and Health Protection of the Institute of Nuclear Research. Apart from the radiation sensitivity difference, the model revealed other fascinating features: inverse cross-sensitivity to UV-C radiation and hydrogen peroxide: the radiation resistant LY-R subline was more UV-C sensitive and hydrogen peroxide sensitive, than the radiation sensitive LY-S subline [2, 3, 7]. This unique pattern of sensitivities is shown in Table 1.

Table 1: The sensitivity pattern of LY sublines.

Sensitivity to	LY-R subline	LY-S subline
X/γ-radiation	low	high
UV-C	high	low
Hydrogen peroxide	high	low

In the passing years many mutants of mammalian cells were obtained and characterised; they became a perfect tool in research that has expanded our knowledge on cellular response mechanisms that act in mammalian cells with damaged DNA. The L5178Y model served the same aim. Below, explanations are given to the sensitivity pattern of LY cells.

Differential sensitivity of LY sublines to hydrogen peroxide

The difference depends primarily on the differential control of iron homeostasis [13]. This is the reason for the difference in iron content, especially that present in the labile iron pool, which is potentially active in the Fenton reaction. In consequence – there is a substantial difference in the initial DNA damage and in the lethal effect of hydrogen peroxide treatment (at micromolar concentrations) [3, 7, 11]. The status of the anti-oxidant defence: thiol content and activity of catalase and glutathione peroxidase contribute to

the difference, whereas activation of NFκB in the pair of LY sublines corresponds with the anti-oxidant status [4].

Difference in UV-C sensitivity

The difference is related to DNA repair abilities: in contrast with LY-S cells, the UV-C sensitive LY-R cells are unable to carry out the nucleotide excision repair [12]. The result is an enhanced sensitivity of LY-R cells not only to UV-C radiation but also to anti-tumour platinum complexes.

Difference in radiosensitivity

Although it is clear that the reason for radiation sensitivity of LY-S cells is an impaired DNA double strand break repair [5, 14, 16] the molecular defect is unknown. These cells exhibit a unique feature: not only the sparing effect of X-ray dose fractionation is missing, but the first radiation dose seems to sensitise to the second one, as survival after divided radiation dose is *lower* than that after single dose [2]. As summarised in Table 2, the sublines exhibit a differential ability to carry out DNA double strand break repair. DNA-PK (DNA-dependent protein kinase) is present in cell extracts (unpublished data). Nevertheless, the repair system impaired in LY-S cells is that of non-homologous end-joining dependent on DNA-PK [15]. There is a possibility (cf [6]) that part of the Ku subunit pool is sequestered in the cytoplasmic compartment, so that its transport to the nucleus is impaired in LY-S cells. Both sublines carry a heterozygous mutation in Tp53 [8], but they differ in propensity to post-irradiation apoptosis: LY-S cells die by apoptotic death in a higher percentage [9].

Table 2: Response of LY cells to X/γ-rays.

Characteristic feature	LY-R subline	LY-S subline
Radiation sensitivity (cloning)	low	high
Effect of irradiation with divided dose	sparing	sensitising
Repair of DNA single strand breaks alkaline elution	at the same rate and to the same residual level	
Repair of DNA double strand breaks neutral elution	normal	impaired
Initial base damage (gas chromatography)	low	high
Repair of base damage (gas chromatography)	at approximately the same rate and to the same residual level	
Activity of DNA-PK in cell extracts	present	

Telomere length – a potential radiosensitivity marker

Another feature of LY sublines is a 7-fold reduction in telomere length in the radiosensitive subline as compared with the resistant one [10], whereas the telomerase activity is similar. Since double strand break repair proteins seem to be involved in the maintenance of telomeres, and several radiosensitive cell lines have short telomeres, the telomere length may prove to be a useful radiosensitivity marker with potential clinical and radiological protection applications.

Conclusions – Outlook

Altogether, the analysis of sensitivity of LY sublines to DNA damaging agents shows that DNA repair is the main important factor that determines the response to the damage. Nevertheless, many additional factors shape the sensitive or resistant phenotype. Their understanding is important both for radioprotection and the improvement of cancer radiotherapy. The recent developments in biology show that much remains to be done.

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