Letter to the Editor

Genotypical instability in undifferentiated cells: precursors for environmental adaptability?

Dear Editor,

With the advent of apoptosis, consisting of a genotypically controlled (and therefore inherited) mechanism of self-destruction, little place is left for stress induced favorable mutational events. Yet evolutionist theory dictates that genotypical variations must be operative. Indeed, the loss of a latent self destruction mechanism, primed to react in the face of stress and therefore serving to preserve an undamaged genotype, would allow for greater mutational variability to persist and hence maintain environmental adaptability. An example of such a situation is observed by the resistance to antitumor therapies of immature myeloid leukemic cells, compared to the more mature compartments.[1] Since it is the progenitor-like neoplastic cell which is responsible for maintaining and replenishing the tumor mass (post-therapy relapse),[2] it would indeed be to its advantage to resist or adapt to stress factors.

Through the use of fluctuation analysis experiments[3–5] using ionizing radiation (IR) as a stress factor and the myeloid leukemia cell lines HEL (CD34+, CD41+, glycophorin A+) and U937 (CD34−, CD41−, glycophorin A−) as representative of two distinct differentiation stages (immature and mature; respectively),[6,7] one can observe that resistance to IR arises spontaneously within the clonogenic fraction of both myeloid leukemia cell lines, but that the occurrence of resistant variant clones in immature cells (HEL) is greatest compared to a more differentiated model (U937) (see Table 1). Resistance did not affect the clonogenic growth rate compared to non-irradiated controls (i.e., clone size was similar). The data presented shows the variability of spontaneously IR resistant clones in eight distinct HEL and U937 populations. Determining the spontaneity or non-spontaneity of a

given event by fluctuation analysis implies that the number of resistant colonies that arise within a population will depend on the rate of mutation and the time of appearance of the variant cell.[8] Moreover, the clones that are scored must arise during the expansion of the parallel cultures. If a pre-existing variant were present in the initial 200 cells in our cultures, with a generation time similar to the parental cells, its contribution to the number of colonies scored after seven generations of growth would be 128 progeny. The highest number of colonies in U937 cells (with one exception) in the 1 Gy IR treated populations was 82, implying that the event which enabled cell survival occurred after a minimum of a single growth generation. With higher IR doses the number of colonies decreased with, for example, a high of six colonies in one of the 8 Gy IR treated populations implying that the resistant mutant occurred after the fourth generation of cell growth. As for HEL, the overall increase in colony number suggests that IR mutants originated much earlier, within a minimum of one or two generations.

Comparison within each of the eight irradiated populations, shows significant fluctuation in the number of resistant clones arising. The most striking fluctuation is seen in the 1 Gy IR U937 group, where the variance is over 19-fold greater than the mean number of surviving colonies, compared to about threefold in the 1 Gy IR HEL group. This implies that at the lowest tested dose of IR, HEL cells were more prone to the occurrence of a spontaneous mutation leading to IR resistance. If resistance were acquired by epigenetic mechanisms, the number of surviving colonies would be expected to have a Poisson distribution, with the variance equal to the mean.[3] Variance

Table 1 Fluctuation analysis of IR-treated HEL and U937 variants

Ionizing radiation dose intensity (Gy	HEL					U937				
	1	2	4	6	8	1	2	4	6	8
Plate no.										
1	156	179	72	54	22	52	15	11	1	2
2	163	148	115	57	31	57	23	13	4	5
3	148	158	113	63	24	65	32	17	4	6
4	214	174	110	47	22	82	29	24	0	3
5	193	168	146	60	36	13	22	10	10	2
6	161	193	133	69	28	66	13	13	8	3
7	169	142	108	63	27	40	32	8	5	5
8	198	200	123	41	29	134	14	16	4	2
Mean colonies*	175.2	170.2	115.0	56.7	27.4	63.6	22.5	14.0	4.5	3.5
Mutation rate[†]	1.7×10^{-2}	1.7×10^{-2}	1.1×10^{-2}	5.6×10^{-3}	2.7×10^{-3}	1.3×10^{-2}	4.6×10^{-3}	2.9×10^{-3}	9.0×10^{-4}	7.0×10^{-4}
Variance	548.5	420.2	468.0	84.2	22.8	1228.3	63.1	25.1	10.9	2.6

*Five groups of eight separate wells were seeded with HEL or U937 cells at a low density (200 cells) and allowed to grow to approximately 3×10^4 cells. The cells from each group were then irradiated at 1, 2, 4, 6, or 8 Gy and then plated on methyl cellulose. All surviving clones (5 to 20 cells) were scored under an inverted microscope after 7 days. [†]Calculated according to Capizzi and Jameson (1973).

at higher IR doses decreased for both HEL (linearly) and U937 (inverse exponential), demonstrating the limitations of spontaneous mutational events (especially for U937 cells) leading to IR resistance at high dose.

IR surviving clones represent descendants from a single mutational event in each population, and is not induced but arises spontaneously with an apparent mutation rate between 1.7×10^{-2} and 7.0×10^{-3}. These mutation rates are high compared to the rate of point mutations described for mammalian cells ($10^{-7} - 10^{-8}$), and to that described for gene amplification ($1 - 7 \times 10^{-5}$).[9-11] It should be noted that these rates are the result of both inherent IR resistance and susceptibility to apoptosis. It could, of course, be argued that the HEL and U937 cell lines, being of leukemic origin, could be more prone to undergo spontaneous or induced genetic recombination. Insofar as general or random mutations are concerned, this is probably a correct assumption, and the known karyotypic instability of some malignant cell lines in long-term culture is evidence that clonal evolution and succession is a common phenomenon in selected growth conditions.

Nevertheless, the present data suggests that HEL cells have a predisposition to spontaneously present survival capacities against an external stress factor. Of course, one can not preclude that these observations are due to differences in the cell lines used rather than to differentiation. However, similar observations were also observed with two other immature myeloid cell lines, KG1 and TF-1-34, compared to the more differentiated U937 and TF-1-33 cell lines (data not shown), underlining the apparent death-resistant nature of progenitor-like myeloid leukemia cells.[12] The origin of this phenomenon is unclear. Leukemia cell lines have, of course, numerous alterations of proto-oncogenes and tumor suppressors that could influence this phenotype. Mismatch repair-deficiency, for example, can lead to an increased rate of spontaneous mutations at selectable loci.[13,14] Secondary alterations of replication fidelity, other repair genes, or genes regulating the cell cycle may further enhance this mutator phenotype. Microsatellite variations also reflect genomic instability, and in some cancers occur concomitantly with mutations in mismatch repair genes.[15] Furthermore, there appears to be a significant background level of transcriptionally productive hybrid gene formation in these cells, as demonstrated by the detection of different types of specific fusion transcripts in the absence of exposure IR.[16] The inherent genomic instability in cells may also be compounded in these cells by being mutant for p53.

Although hematopoietic cell lines reflect hematopoiesis occurring *in vivo* to only a limited degree, it is possible to extrapolate. In programmed cell death, the protection of a gene pool is coded for. This suggests that apoptosis was part of a primitive event, and to have been inherited or the result of convergent evolution. The latter would imply that all organisms capable of apoptosis are at an evolutionary dead end and apoptosis will preserve that organism as it is. However, such a rationale may not be extrapolated to progenitor or stem cells, be they neoplastic or normal. Indeed, surviving environmental stress suggests that genotypical variations are a necessity. The greater occurrence of spontaneously resistant variant clones to IR in immature leukemic cells, may reflect a predisposition (or eventually a programmed mechanism) for survival at all costs, congruant with environmental 'adaptability' and even 'evolvability'. This greater genetic instability of progenitor-like cells is illustrated here in myeloid leukemia cells and could be the basis for secondary leukemias and myelodisplastic syndromes attributed to IR- and chemo-therapy.

Alain P Bruno[1], Guy Laurent[1,2], Cécile Demur[2] and Jean-Pierre Jaffrezou*,[1]

[1] INSERM E9910, Institut Claudius Régaud, Toulouse, 31052, France
[2] Service d'Hématologie, Centre Hospitalier Universitaire Purpan, Toulouse 31059, France
* Corresponding author: INSERM E9910, Institut Claudius Régaud, 20 rue du Pont St Pierre, Toulouse, 31052, France E-mail: jaffrezou@icr.fnclcc.fr.

1. Ploemacher RJ *et al.* (1992) Int. J. Radiat. Biol. 61: 489 – 499
2. McCulloch EA. (1986) J. Cell Physiol. 4: 27 – 33
3. Luria SE and Delbruck M. (1943) Genetics 28: 491 – 511
4. Law LW. (1952) Nature 169: 628 – 629
5. Goldie JH and Coldman AJA. (1979) Cancer Treat. Rep. 63: 1727 – 1733
6. Bailly JD *et al.* (1995) Leukemia 9: 1718 – 1725
7. Bailly JD *et al.* (1997) Leukemia 11: 1523 – 1532
8. Kendal WS and Frost P. (1988) Cancer Res. 48: 1060 – 1065
9. Tlsty TD *et al.* (1989) Proc. Natl. Acad. Sci. USA 86: 9441 – 9445
10. Cole J *et al.* (1976) Mutat. Res. 41: 377 – 386
11. Crawford BD *et al.* (1983) Mol. Cell. Biol. 3: 931 – 945
12. Bruno AP *et al.* (1998) Cell Death and Differ. 5: 172 – 182
13. Eshleman JR *et al.* (1995) Oncogene 5: 33 – 37
14. Phear G *et al.* (1996) Mol. Cell. Biol. 16: 6516 – 6523
15. Fishel R and Kolodner RD. (1995) Curr. Opin. Genet. Dev. 5: 382 – 395
16. Deininger MWN *et al.* (1998) Cancer Res 58: 421 – 425