Radiosensitivity, radiation-induced cancers and the Chernobyl disaster

Measuring radiosensitivity is a major aim of radiation biology. A range of functional aspects of radiosensitivity has been investigated, mostly to optimize patient selection and dose tuning in the context of cancer radiotherapy [1,2]. The present position paper addresses questions covering other areas. Can one find out whether the cancer developing within a particular individual was caused by radiation? Can one measure the propensity of a given healthy individual to develop cancer following radiation exposure? The Chernobyl disaster opened a window on this matter.

An increased incidence of thyroid carcinomas in children was first noticed in Belarus and Ukraine 4 years after the 1986 Chernobyl accident [3,4]. Increased incidence has been observed since then in people exposed to fallout during childhood in these regions [5,6]. The aggressiveness and morphology of these tumors, over 95% classified on the basis of their pathology as papillary thyroid carcinomas (PTC), appear to be related to the age of patients at the time of the accident and the lag between the accident and the diagnosis, that is, the latency of the cancers [7]. Given the uncertainties regarding the variability of latency with dose, individual’s age at exposure, lifestyle and genetic background, it seems questionable at this point to estimate how many cancers will develop as a result of the accident [8]. Nevertheless, incidence data collected so far support the notion that only a small fraction of the several hundred thousand inhabitants of the most heavily exposed area of Belarus and Northern Ukraine is likely to develop a PTC because of radiation. This is of obvious relevance to measure the propensity of a given healthy individual to develop cancer following radiation exposure.

Microarrays profiling: damage versus susceptibility models

The response to radiation involves a number of protection and repair mechanisms, including the clearance of reactive oxygen species and the repair of DNA double-strand breaks. These are only partially assessed by any one of the current radiosensitivity assays [1]. Which biological function and assay is the most relevant to human cancer initiation is unknown. Instead of focusing on any particular biological function, gene expression microarrays measure the molecular phenotype of tissues on a genome-wide scale. The microarray profile of a tissue contains traces of its cell type composition and all the processes controlled by or feeding back on mRNA
transcription and decay in these cell types. This open-endedness, together with the possibility of applying them seamlessly to in-vitro system and in-vivo tissues, make microarrays particularly attractive to address the questions posed previously.

Gene transcription depends on environmental factors and DNA features such as copy number, mutational state, methylation, etc. Post-Chernobyl PTCs were most likely induced by the $^{131}$I concentrated in the thyroid, which has a half-life of 8 days. Although DNA damage is typically repaired within a time scale of hours, the tumor material available for analysis has typically been surgically extracted more than a decade after the 1986 accident. Thus, any gene expression signature discriminating post-Chernobyl from spontaneous cancers must have been sustained over this time interval. Consequently, either the signature is a ‘damage signature’, that is, it is a late result from radiation-induced DNA damage (nonrepaired or incorrectly repaired damage) or it is a ‘susceptibility signature’, that is, it mirrors radiation susceptibility factors preexisting to the accident or both. Thus, microarrays have the potential to address the questions posed previously but may not directly distinguish a damage from a susceptibility model.

**RET/PTC rearrangements versus BRAF mutations**

The early research on post-Chernobyl PTC focused on a damage model. The vast majority of PTCs harbor either a BRAF mutation (45%, [9]) or a rearranged in transformation/papillary thyroid carcinoma (RET/PTC) rearrangement (35% in adults, [10]), which are generally mutually exclusive [11]. Several research teams have reported higher frequencies of RET/PTC rearrangements in post-Chernobyl PTCs [12]. In line with the damage signature model, these higher frequencies could result from the fact that radiation induces double-strand breaks, and thus rearrangements rather than point mutations [13].

The induction of RET/PTC rearrangements after in vitro irradiation of immortalized thyroid cells [14] would support the former explanation. Both gene alterations result in the constitutive activation of the RAS–RAF–mitogen-activated protein kinase signaling pathway [11,15]. Nevertheless, gene expression signatures separating BRAF from RET/PTC tumors have been reported. The number of genes involved, however, varies from a few dozen [16] to several thousands [17]. Array-CGH, which measures DNA copy number genome wide at high resolution, has revealed distinctive chromosomal aberrations in RET/PTC-positive compared with RET/PTC-negative tumors [18].

Although early reports pointed toward a lower BRAF mutation frequency in post-Chernobyl patients, recent evidence suggests that the BRAF mutation is associated with age and is more prevalent among older post-Chernobyl patients or patients with longer latency tumors or both [19–21]. Moreover, $\text{H}_2\text{O}_2$, which is produced in large amount during thyroid hormones synthesis, also causes double-strand breaks in thyroid cells (Driessens et al., personal communication). Thus, more research is needed to confirm the association of RET/PTC with radiation. Regardless of the outcome of this research, the fact that 35% of spontaneous PTCs do harbor a RET/PTC translocation [10] sets the limit on the specificity of this marker as a detector of radio-induced cancers.

**Molecular signatures in post-Chernobyl papillary thyroid carcinomas**

The first microarray profiling study of post-Chernobyl PTCs did not uncover any significant difference with PTC from French patients without documented history of exposure to radiation [22] – no PTC incidence peak was observed in France following the 1986 accident [23]. This supports with molecular evidence the pathology finding that both are instances of the same disease. This early study, however, analyzed 2000 of the 21000 genes present in the human genome.

Port et al. [24] pooled and hybridized on full genome arrays the mRNA of 10 post-Chernobyl and 10 spontaneous PTCs. Several hundreds of genes were differentially regulated between the two groups. Because a single aberrant sample could dominate the content of an entire mRNA pool, they confirmed the expression of 92 genes on independent individual samples by reverse transcriptase-PCR. A functional classification of the expression signature revealed an underrepresentation of genes associated with the immune response and an overrepresentation of genes coding for G-proteins, oxdoreductases and growth factors. These features were interpreted as correlates of the generally higher aggressiveness of post-Chernobyl PTCs.

Our group recently hybridized 12 post-Chernobyl PTCs from Northern Ukraine and 14 PTCs from France on microarrays covering 8000 genes [25]. Unsupervised classification on the basis of the entire set of genes confirmed that, when considered on a global scale, the two types of cancers could not be distinguished. Nevertheless, a supervised analysis uncovered subtle expression differences. A cross-validation designed to exclude overfitting and selection biases established that post-Chernobyl tumors were detectable with a specificity of 93% and a sensitivity of 85%. These numbers are impressive when viewed in the context of the notoriously challenging thyroid pathology [26]. This and findings from the study by Port et al. [24] suggest a positive answer to the question of whether the cancer
developing within a particular individual was caused by radiation.

What is the biological meaning of this classifier? The BRAF and RET/PTC genetic alterations are not the explanation as they were not significantly associated with the radiation status of the tumors. Remembering that most of the exposed population did not develop PTC after the accident, we aimed instead at characterizing our signature in terms of a susceptibility profile. A growing body of evidence suggests that, in the absence of radiation, endogenous H₂O₂ is a likely PTC-initiating factor [27,28]. We reanalyzed published transcriptional responses of lymphocytes to various toxic agents in vitro and found that, among 10 genotoxic agents, H₂O₂ elicited the response most closely related to that of radiation, suggesting that they cause similar damages to the cells [29]. Apart from this similarity, however, 118 genes were found to be differentially expressed by a factor of more than 1.5 between the H₂O₂ and γ-radiation responses and were sufficient to sort post-Chernobyl apart from spontaneous tumors with high accuracy. Hence, a link between in-vivo tumors and in-vitro assays characteristic of their putative etiological agent could be established. Furthermore, we derived five signatures for the five major repair pathways from the literature on DNA repair. One of them, composed of 13 homologous recombination genes, accurately classified the tumors according to their origin. The probability that 13 randomly selected genes achieve this feat is extremely low. Homologous recombination repairs double-strand breaks. Although these results do not rule out a damage model, they support a susceptibility component in the post-Chernobyl signature. Whether this susceptibility is inherited and whether it is measurable in healthy cells of any type (e.g. blood cells) remain to be investigated.

Our study, the one by Port et al. [24] and most early RET/PTC studies suffered from the unavailability of age-matched and ethnicity-matched patients. Are the reported differences due to the radiation status of the tumor or to these potential confounders? Our functional characterization suggests that radiation is the explanation; if not, one would have to explain why homologous recombination, but not four other repair mechanisms, depends on age or ethnicity or both. The Chernobyl Tissue Bank (www.chernobyltissuebank.com) has now accumulated tumors from young patients born after 1987. Ongoing studies will formally settle the issue.

**Are post-Chernobyl patients more susceptible to radiation?**

The classification of post-Chernobyl and spontaneous tumors on the basis of the 118 H₂O₂ vs. γ-radiation genes suggests that radiation-induced cancers arise in patients more sensitive to radiation and ‘spontaneous’ cancers in patients more sensitive to the effect of H₂O₂. However, susceptibility to H₂O₂-induced carcinogenesis also depends on factors acting upstream of the DNA damage response such as H₂O₂ production, removal and intracellular localization [27,28]. Let us envision, for the sake of reasoning, two extreme theoretical models. In model A, these upstream factors determine entirely the development of spontaneous PTCs, whereas in model B, they do not play any role; the same DNA damage-related factors initiate radiation-induced and spontaneous tumors (Fig. 1). Model B has the counterintuitive consequences that post-Chernobyl patients should be, on average, ‘less’ radiosensitive than their spontaneous counterpart; it takes the cumulative damage incurred by H₂O₂ and radiation to induce a cancer, whereas only H₂O₂, that is, less DNA damage, is required to induce spontaneous cancers. The truth most certainly lies between models A and B. Nevertheless, in agreement with model B, Xiong et al. [30] demonstrated that the number of chromatid breaks per cell following γ-irradiation was significantly higher in the lymphocytes of 57 PTC patients with no documented exposure to radiation than in the lymphocytes of healthy controls, suggesting that radiosensitivity may favor spontaneous PTC initiation. This difference could be related to impaired homologous recombination, as the 18067T allele variant of XRCC3 was more frequent in 134 thyroid cancer patients than in 166 healthy individuals in another study [31]. Quantifying the involvement of the radiation response in spontaneous PTC patients will reveal to what extent post-Chernobyl signatures operate by distinguishing between unrelated mechanisms (model A) or by distinguishing quantitatively the efficiency of the same DNA repair system among the patients (model B). The scope of the signatures depends on it: are they quantitative measures of a general PTC susceptibility or are they specific for radio-induced PTCs? Further, are they applicable in the context of other cancers apart from PTC and possibly other radiation-related diseases?

**Related work**

The susceptibility model is supported by the finding that different TP53 alleles are associated with radiation exposure in PTCs from Russian–Ukrainian patients [32]. Further, primary malignancies associated with thyroid cancer involve distant anatomical sites including adrenal glands, bones and kidneys [33], supporting a cancer susceptibility spanning different organs.

An increased radiosensitivity is associated with increased cancer incidence in genetic syndromes such as ataxia telangiectasia [1]. Is this unique to these rare syndromes, or are these syndromes the extremes of variability in oncologically relevant radiosensitivity in the general
population? The microarray profiles of lymphocytes drawn from the unaffected parents of patients with retinoblastoma could be distinguished from those of the parents of healthy controls [34]. Cells from parents of patients with retinoblastoma were also found to be hypersensitive in a low dose-rate γ-H2AX assay [35].

Another study surveyed more than 10,000 individuals irradiated at high dose on the scalp for the treatment of tinea capitis [36]. An increased incidence of meningiomas in the cohort and a nine-fold increased familial clustering of the disease were reported, again suggesting an inherited susceptibility. Other cancers, including thyroid cancers, are more frequent in this cohort and are more likely among the siblings of patients with meningiomas.

**Conclusion**

Thus, several lines of evidence suggest that the propensity to develop cancer following radiation exposure is variable in the general population and could be measurable from gene expression. Many questions are pending and we are far from a properly validated test, yet it is our duty to think about the potential societal impact of this research.

Radiation is used in various diagnostic and therapeutic procedures. A cancer radiation-susceptibility test could help select the patients or tune radiation doses or both. The same concept could be applied to exposure in the...
work place, although this would raise several ethical and legal issues. Our personal opinion is that regulatory discrimination of workers is questionable; exposure must instead be reduced.

The research presented here supports the feasibility of a test assessing whether a given tumor resulted from radiation exposure, but its precision remains to be determined. Yet, it may be possible to measure the prevalence of a specific radiation susceptibility profile in a group of individuals. This could have consequences in the context of class action lawsuits. It may also have a bearing on epidemiological investigations of rare cancers. Instead of assessing disease incidence in huge groups – for example, Cardis et al. [37] surveyed 500,000 nuclear plant workers – one could assess the incidence of the susceptibility profile only in the much smaller and tractable group of cancer patients. If radiation initiated cancers in a population, a higher fraction of the cancer patients from that population should be positive for the radiation susceptibility signature.

Current radioprotection policies rely on the linear no-threshold model assuming that radiosensitivity is uniform across populations and that radiation-induced carcinogenesis strikes individuals randomly. The existence of variation in radiation susceptibility and possibly of different susceptibilities in different populations may challenge this model.

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References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
•• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 476).


This study shows an increased radiosensitivity of the lymphocytes of patients who spontaneously developed thyroid cancer.


This study demonstrates an increased radiosensitivity of the unaffected parents of patients with retinoblastoma.


This study demonstrates a familial clustering of meningiomas in a large cohort of irradiated individuals.