An Assessment of Low-dose Health Effects of A-bomb Radiation by Neural Networks Theorem

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Cancer risk at low doses of ionizing radiation remains poorly defined due largely to the ambiguity at low doses in A-bomb survivors stemming from limitations in statistical power and information available on overall radiation dose. To deal with these difficulties, a novel nonparametric statistics system was developed based on the "*integrate-and-fire*" model of artificial neural networks (ANN) and tested on cancer databases of A-bomb survivors which were publicly available from Radiation Effects Research Foundation (RERF). The analysis revealed a small but significant elevation of cancer risk at low doses in Nagasaki survivors, which hampered the precise estimate of cancer risk at low doses. When Hiroshima survivors were treated separately, the analysis disclosed a threshold with upper dose limit of 0.1-0.2 Sv, which varied with cancer site, gender and age at exposure. Curiously, the threshold was manifested as a negative excess relative risk, or a reduction of spontaneous cancer rates. Furthermore, such threshold was also observed in cardiovascular diseases, suggesting that cancer and atherosclerosis may entail fundamentally common biological mechanisms.

In a single perceptron model of ANN, the '*integrate-and-fire*' response is described by

$$S(x) = \varphi\{\sum w_i \lambda(x_i) - \mu\} \quad , \qquad (1)$$

where $\varphi(..)$ is a gain function, $\lambda(x_i)$ is the ith input variable with weight w_i and μ is threshold (McCulloch and Pitts, Bull. Math. Biophys., 5:115, 1943). In the implementation of the ANN theorem into cancer risk assessment, the relative risk (*RR*) of cancer at dose x, $\lambda(x)$, was regarded as an input variable and we dropped the threshold term but left to be automatically determined *in situ* because the threshold was not known *a priori*. The discrete function of the weighted sum of *RR* thus given in Eq. 1 was fitted by aid of AIC, ML and bootstrap to a continuously differentiable polynomial function, $\Psi_{RR}(x)$, which was then differentiated to obtain continuous probability density function of relative risk, $RR(x)=\Psi_{RR}'(x)$, or excess relative risk, $ERR(x)=RR(x)-1$. When conventional piecewise dose category method as adopted by Preson *et al.* (Radiat. Res., 168:1, 2007) and present ANN method were compared for the excess relative risk (*ERR*) of solid cancers in combined Hiroshima and Nagasaki cohort, the two methods gave very comparable results including an abnormal elevation at low doses as previously noted by Pierce and Preston (Radiat. Res., 154:178, 2000) (Fig. 1). When the ANN analysis was performed in two cities separately, it became evident that the abnormality at low dose was solely due to cancer of lung, liver and gallbladder in Nagasaki survivors. Its city- and organ-specificity suggests an involvement of internal deposit of plutonium in some distally exposed survivors. Indeed, fallout of $^{239/240}$Pu has been detected in the East to the hypocenter (Saito-Kokubu, J. Geosci. Osaka City Univ., 50:7, 2007). Avoiding disturbance by yet unproven origin of the low dose abnormality in Nagasaki, the representative dose response of *ERR*

was determined in Hiroshima survivors alone, which was Y_H=(0.584±0.018)Sv+(0.040±0.001)Sv^2.

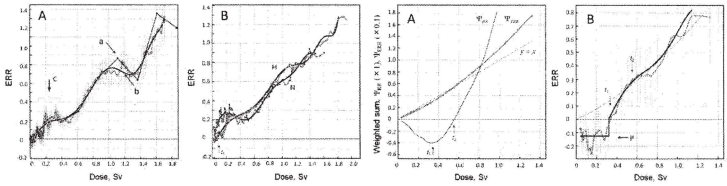

Fig. 1 **Fig. 2**

Fig. 1. The *ERR* of solid cancer. **A**: Cross-validation of the statistics methods; conventional piecewise dose category method (a) and ANN method (b). Abnormal elevation of *ERR* at low doses is shown by arrow (c). *ERR* data obtained by moving window averaging are shown by open circles together with 80% CI. **B**: Comparison of *ERR* between Hiroshima (H) and Nagasaki (N) as assessed by ANN method. Abnormality at low doses is evident in Nagasaki.

Fig. 2. Methodological representation in a sample cohort, *i.e.*, lung cancer in Hiroshima male survivors exposed at 20 yrs or older at the time of bombing. **A**: Weighted sum and optimization by fitting to continuous function of dose. $\Psi_{ERR}(x)=\Psi_{RR}(x)-x$. **B**: Dose-dependent probability density function of *ERR* as determined by $\Psi_{ERR}(x)=\Psi_{RR}(x)-1$. Dotted line: Dose-response independent of the threshold response ($x>t_2$), Y=(0.359±0.066)Sv+(0.219±0.066)Sv^2.

A significant finding was the presence of threshold in some cancer. Unexpectedly, the threshold was identified as a negative *ERR*, or a reduction of the spontaneous cancer rate (Fig. 2). The integrated *ERR*, Ψ_{ERR}, decreased linearly with dose at low doses then increased with further increase of dose. The threshold dose, t_1, was determined as the dose that satisfied the derivate Ψ_{ERR}'=0 by numerical differentiation of Lagrange. The linearly decreasing part ($x \leqq t_1$) was fitted to a linear regression $\Psi_{ERR}=a+\mu x$, the derivation of which gave the *ERR* at threshold, *ERR*=μ. The dose, t_2, at which Ψ_{ERR}=0, corresponds to the dose thereafter all survivors in a moving block have a non-threshold dose $x>t_1$. Thus, the *ERR* is discontinuous with a breakpoint at t_1, followed by a transient phase $t_1<x<t_2$, and then the dose range that is free of the threshold response $x>t_2$, where a fundamental dose-response may be calculated (Fig. 2b).

The threshold response was dependent on cancer site, gender and age at the time of bombing (ATB). Considering variability of absorbed radiation dose according to the variability in organ (target) size and distribution in the body, the relevant dose appears to be below 100-200 mSv. When appeared, its magnitude was generally 10-20 % reduction of spontaneous frequency, *i.e.*, μ=-(0.1~0.2). It was prominent in cancer of lung, stomach, liver, gallbladder, pancreas and prostate in male survivors with age ATB>20 yrs. The threshold was not noticeable in female cancer except for liver and breast cancer. As a consequence, the threshold was clearly seen in male solid cancer combined whereas it was small, if any at all, in female survivors (Fig. 3 left panel). It should be noted that there was no radiation effects, neither in its reduction nor in promotion, on the development of cervical cancer (Fig. 3 right panel). The refractoriness of cervical cancer to radiation could explain a direct role of functional inactivation of p53 and RB1 tumor-suppressor proteins by papillomavirus (HPV) early antigens, E6 and E7.

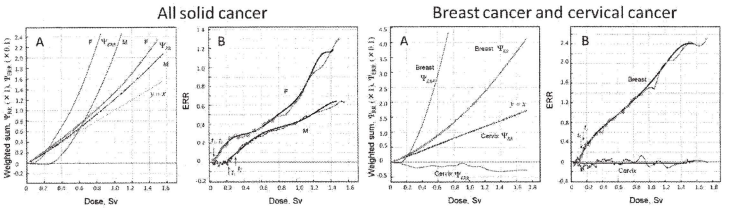

Fig. 3. Characteristics of dose response of solid cancer, breast cancer and cervical cancer (Hiroshima). **A**: Weighted sum of *RR* and *ERR*. **B**: Probability density of *ERR*. M: male survivors. F: female survivors.

The radiation effects on leukemia are presented in Fig. 4. In Hiroshima, both males and females survivors were characterized by the threshold, and the dose responses of *ERR* for non-threshold doses were linear-quadratic, $Y=(0.924\pm0.066)Sv+(0.563\pm0.053)Sv^2$ for males and $Y=(0.089\pm0.147)Sv+(0.737\pm0.104)Sv^2$ for female (Fig. 4 left panel). The linear-quadratic (L-Q) dose response suggests a common mechanism in the causation shared by leukemia and solid cancer, and does not support the hypothesis of clone selection by radiation of pre-existing preleukemic cells (Nakamura, Radiat Res., 163:258, 2005). Response of leukemia in Nagasaki differed from that in Hiroshima. In addition to being superimposed by abnormal elevation at low doses like in solid cancer, the reduction of *ERR* at moderate doses seen in female survivors was not seen in male survivors, where *ERR* continued to increase with dose (data not shown). However, when analysis was made after exclusion of factory workers, both sex showed very comparable response patterns (Fig. 4 right panels).

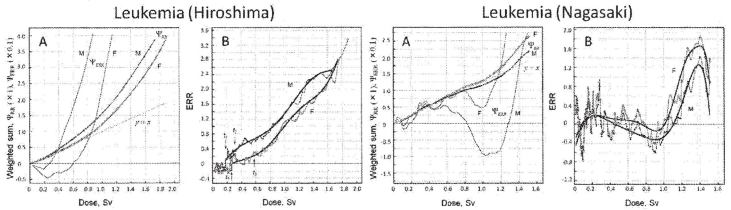

Fig. 4. The dose responses of leukemia in Hiroshima and Nagasaki. In Nagasaki survivors, factory workers have been excluded. **A**: Weighted sum of *RR* and *ERR*. **B**: Probability density of *ERR*. M: male survivors. F: female survivors.

In Nagasaki survivors, about 17 % of leukemia is adult T-cell leukemia (ATL), in which HTLV-1 retrovirus plays a causative role in its development. ATL is endemic to South-West part of Japan including Nagasaki. The observations suggest that most factory workers are not Nagasaki natives and that radiation suppressed the clinical manifestation of ATL in HTLV-1 carriers, giving rise to the negative *ERR* at low doses. This unique response could be the reason of purely quadratic function of dose previously reported for leukemia in Nagasaki survivors.

When the ANN statistics was applied to noncancer diseases, the threshold response was also found in cardiovascular diseases but not in other noncancer diseases tested, indicating that the cardiovascular diseases and cancer may entail common biological mechanism. The mechanism of threshold response at

low doses remains to be elucidated. Yet, it is tempting to correlate with recently growing evidence for the pathway choice in the repair of DNA double-strand break (DSB). The DSBs are the subject to be repaired either by restitutional non-homologous end-joining (C-NHEJ), or mutagenic alternative end-joining (Alt-NHEJ) or homologous recombination (HR) including single-strand annealing (SSA). C-NHEJ is activated by low-dose radiation, which in turn suppresses Alt-NHEJ and HR (Liever *et al.*, Nat. Rev. Mol. Cell Biol., 4:712, 2003; Mladenov and Iliakis, Mut. Res., 711:61, 2010). The DSBs generated at DNA replication stalled at fork triggered by DNA damage associated with exogenous or endogenous genotoxin are also the subject to be repaired by C-NHEJ, Alt-NHEJ or HR. The activation of C-NHEJ by low-dose radiation (Tachibana, Adv. Biophys., 38:21, 2004; Klammer *et al.*, Cancer Res., 2010), and hence suppression of Alt-NHEJ and HR, may eventually suppress the mutagenic process and thus suppresses spontaneously occurring cancer rate.

Prevalence of threshold in male survivors exposed at adulthood suggests the involvement of smoking in spontaneous cancer and its suppression by low doses. It is also known that C-NHEJ or deficiency in Alt-NHEJ suppresses the integration of retroviral DNA into host DNA. This may account for the suppressive effect on ATL at low doses. Long-term, or life-time long, sustainability of low dose effects *in vivo* has been suggested by Kakinuma *et al.* (J. Radit. Res, 50:401, 2009) for the suppression of spontaneous and chemical-induced tumor in mice pre-irradiated with low dose X-rays. Altogether, the present finding brings about a new paradigm in radiation protection and risk assessment where low-dose health effects are not a simple stochastic process against radiation but an integrated consequence of highly ordered biological response of the genome to threat.

The present ANN statistics was conceived, programmed by MSS and tested on the cancer databases released from RERF. The RERF is a private foundation funded equally by the Ministry of Health, Welfare and Labor of Japan and the U.S. Department of Energy through the U.S. National Academy of sciences. The conclusions in this report are those of the author and do not necessarily reflect the scientific judgment of the RERF or its funding agencies.