Molecular basis of basal cell carcinogenesis in the atomic-bomb survivor population: p53 and PTCH gene alterations

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Introduction

Skin cancer is a common cancer, accounting for about half of all cancers in the United States, with BCC constituting 80% of all non-melanoma skin cancers (1). However, skin cancers are relatively rare in Japan, with currently estimated background incidence rates being ~3 per 100,000 each for BCC and SCC (2). These rates are much lower than those of ~200 for BCC and ~40 for SCC in US whites (1). Nevertheless, the BCC rates have been increasing rapidly in Japan (2), although the current rates have not reached the level (~30 per 100,000) seen in the Japanese residents of Kauai, Hawaii (3). The major cause of skin cancer is not only UV but also other etiological factors exist.

Elevated risks of skin cancer following exposure to ionizing radiation (IR) for medical reasons have long been known (4). The Life Span Study (LSS) cohort of atomic bomb (A-bomb) survivors has been followed up after the A-bomb explosions in 1945 in Hiroshima and Nagasaki. Previous reports from the LSS cohort indicate that the risk for nonmelanoma skin cancer is radiation dose dependent (5). Interestingly, this excess for skin cancer is primarily driven by the excess relative risk (ERR) at 1 Sv of 1.8 for basal cell carcinoma (BCC) whereas there is no evidence of radiation effects on genesis of squamous cell cancer or melanoma of the skin (6). In general, all major types of skin cancers, including BCC, are strongly associated with UV exposure, and a possible interplay of UV and IR exposure in skin carcinogenesis is of special interest (1,4). In the A-bomb survivors, the absolute excess risks of BCC attributable to IR for tumors at body sites usually shielded from UV are similar to those for tumors at sites more frequently exposed to UV (2), suggesting that the effects of UV and IR on BCC may be independent.

Carcinogenesis mechanisms are by no means straightforward but in most cases are likely to be both complex and multi-stepped and dependent upon the accumulation of critical genetic alterations in oncogenes and tumor suppressor genes (7). Some carcinogens are known to leave specific alterations—so-called molecular signatures—in genes. UV is one such carcinogen that induces signature DNA damage that often leads to base substitution mutations in adjacent pyrimidine sequences (8). On the other hand, IR induces a variety of different types of DNA damage, of which the most significant may be double-strand breaks that lead to deletions (9). These differences may provide us with a rationale upon which we can base attempts to distinguish alterations induced by different carcinogens in the target genes for BCC among the survivors. Mutations in the p53 tumor suppressor gene, which plays a major role in the regulation of the cell cycle, apoptosis and DNA repair, are known to be associated with skin carcinogenesis (10,11). Mutation of this gene has been reported in ~50% of BCCs, sometimes affecting both tumor and pre-tumor tissues (12–17). These results suggest that p53

Abbreviations: BCC, basal cell carcinoma; ERR, excess relative risk; IR, ionizing radiation; LSS, life span study; LOH, loss of heterozygosity; NBCCS, nevoid basal cell carcinoma syndrome; Py–Py, pyrimidine–pyrimidine; RERF, Radiation Effects Research Foundation; RFLP, restriction fragment length polymorphism; SCC, squamous cell carcinoma.

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mutations may be important in the earlier stages of skin carcinogenesis. Additionally, some skin cancers have been reported to have different $p53$ mutations within the same tumor (18).

The **PTCH1** gene, located on 9q22.3, has been identified as a human homolog of the *Drosophila patched* gene (19,20). This gene is associated with a hereditary disorder, nevoid basal cell carcinoma syndrome (NBCCS) that predisposes affected individuals to cancers such as BCC and medulloblastoma. Loss of heterozygosity (LOH) of chromosome 9 has also been shown to be a relatively common event in BCC (15,21–23). To evaluate the possible involvement of the **PTCH1** gene and/or the $p53$ gene in radiation-induced BCC development, we carried out molecular analyses of the structure of these genes in cells taken from multiple tumor foci in BCC-affected A-bomb survivors.

### Materials and methods

#### Samples

There were a total of 80 BCCs diagnosed from 1958 to 1987 among 208 persons, who developed skin tumors, of the LSS cohort of ~80,000 survivors followed up by the Radiation Effects Research Foundation (RERF). We obtained surgically removed samples that were formalin-fixed and embedded in paraffin blocks. Among them, we selected 47 BCCs whose individual size and quality were suitable for molecular analyses. These 47 tumors originated from 39 A-bomb survivors; multiple BCC tumors occurred in 5 persons, ranging in number from 2 to 5 (Table I). Some large tumors were examined as two or three separate portions; thus, in total, we examined 72 tumor specimens representing 47 individual BCCs from 39 survivors. The samples were grouped according to whether they were exposed to UV and/or IR (Table I). Subjects with BCC from the high-dose IR-exposed survivors (high-IR: 1 Gy and more) were compared with those from the low-dose IR-exposed survivors (low-IR: <0.2 Gy) (24). The cut-off point of 1 Gy was used because the BCC dose-response curve showed a negligibly small risk below this dose (6). Subjects with high-IR exposure were matched by age, gender and the anatomical site of the body with roughly twice the number of subjects with low-IR exposure. BCCs occurring on the face and hands were defined as high-UV, and BCCs from the rest of the body as low-UV. No matching was made between tumors with high-UV exposure versus low-UV exposure. In all cases the tumors occurred either in high- or low-UV exposed sites of the body. Statistical analysis (see below) considered individual tumors rather than persons.

#### DNA extraction and PCR amplification

All sections used in this study were pathologically confirmed as BCC by a pathologist using hematoxylin–eosin stained tissues. After confirmation, normal and tumor portions were collected separately by scalpel from two 3-μm-thick sections. DNA extraction was carried out as described previously (25). The procedures used for PCR amplification and LOH analyses of the $p53$ gene were as described by Ito *et al.* (26). Evidence of LOH was obtained by analysis of restriction fragment length polymorphism (RFLP) for $p53$, or by analysis of the microsatellite markers, D9S197, D9S280 and D9S287 for the **PTCH1** gene (Figure 3A) (27).

### Table I. The BCC samples grouped according to whether they were exposed to UV and/or IR

| UV (high) | UV (low) |
|---------|---------|
| No. of Tumors/person, tumors persons mean (range) | No. of Tumors/person, tumors persons mean (range) |

| IR (high) | 14 | 8 | 1.8 (1–5)* | 6 | 5 | 1.2 (1–2) |
| IR (low) | 14 | 14 | 1.0 (1–1)* | 13 | 12 | 1.1 (1–2) |

Mean age (y) at IR exposure: 21.3 (high-IR), 37.5 (low-IR). Males: 53.8% (high-IR) and 46.2% (low-IR). *Significant difference with $P = 0.05$, unpaired t-test.

**Sequencing**

All amplified PCR products were directly sequenced by an ALFred automated sequencer (Amersham Pharmacia) using Cy5-fluorescent-labeled primers according to the manufacturer’s instructions.

**Statistical analysis**

For multivariate discrete data analysis, graphical log-linear modeling (28) was performed. Graphical modeling expresses a relationship of some variables in terms of a graph and gives some conditional independence relationship of the variables. Consequently, the advantage of a graphical modeling approach is that it is tractable over other methods; specifically, it provides inference tools to naturally handle situations of missing data entry because of the conditional dependencies encoded in the graph structure and it can easily combine prior knowledge with data in the framework. (29,30) In this study using graphical modeling, there are two kinds of analyses. In the first analysis, graphical log-linear modeling was applied to the variables, F-mt (mutation sequences occurred at pyrimidine–pyrimidine (PyPy), CpG (not adjacent to PyPy) and other sites), UV, IR, T-ex (time elapsed between exposure and diagnosis) and ATB (age at the time of A-bomb exposure). In the second analysis, graphical log-linear modeling was applied to the variables, P-mt (mutation type, such as missense, nonsense and silent), UV, IR, T-ex and ATB. The critical $P$-value from the model selection in graphical log-linear modeling was 0.05.

For this analysis, important variables were IR, UV, ATB and T-ex. The IR variable is defined as two categories: doses <0.2 Gy (low-IR) was designated as 1, and ≥1 Gy (high-IR) as 2. The UV variable was defined as two categories: low exposure (low-UV) was designated as 1 and high exposure (high-UV) as 2. The ATB was defined as three categories: 0–19-years-old was designated as 1; 20–39-years-old as 2, and 40-years-old or over as 3. The time since exposure was calculated after subtracting age at exposure from age at diagnosis, and the time elapsed since exposure variable (T-ex) was defined as two categories: a duration of 0–34 years was designated as 1, and a duration of 35 or more as 2. For $p53$ sequence data, detected mutation (F-mt) was defined as three categories: a mutation at a PyPy site was designated as 1; a mutation at a CpG site (not at a PyPy site) as 2; and a mutation at any other site as 3. For the analysis of expected protein mutation (P-mt), the three designated categories were missense mutations as 1, nonsense mutations as 2 and silent mutations as 3.

**Results**

The background incidence rate for BCC in our cohort was ~3 per 100,000 per year. Table I shows the distribution of cases used in this study according to UV and IR exposure. Notable is the significantly higher average number of tumors per person from high UV- and high IR-exposed areas of the skin compared to the average from high UV- and low IR-exposed areas. Note that high versus low UV exposures are defined by tumor location. Tumors occurring on the usually UV exposed face and hands are considered highly UV exposed while those in the rest of the body, usually shielded from UV, have low exposure. IR exposures are based on DS86 skin doses estimates (24); high-IR tumors were exposed to ≥1 Gy and low-IR tumors, <0.2 Gy.

Most samples were found to be analyzable by PCR-based methods using DNA extracted from formalin-fixed and paraffin-embedded archived tissues. We began by using five different RFLP markers to test for LOH in the vicinity of the $p53$ gene. Of the 47% of the cases that were informative, there appeared to be no difference in LOH between the low-IR and high-IR groups (data not shown).

Amplified PCR fragments of the hot spot exons 5–8 of the $p53$ gene were then subjected to direct sequencing. All the observed mutations were base substitution mutations (mainly C-to-T transitions), and there were no deletions. Among the five survivors with more than one tumor, the multiple tumors within four persons varied as much as tumors in different individuals (data not shown). Table II presents crude frequencies (unadjusted for age, gender or time) of various
types of p53 mutations by IR and UV exposure. The overall frequency of tumors with p53 mutations did not differ significantly by UV or IR exposure, although the proportion of tumors with any mutation was somewhat higher for the high-IR group. However, there were differences in distribution of mutations by type and site between exposure groups (also graphically presented in Figure 1A and B). Thus, for example, there were more silent mutations in the high-UV group than low-UV group.

Since mutation of the p53 gene was affected by various factors, such as age at exposure and time since exposure, a multivariate statistical analysis, using a graphical modeling technique, was performed to assess the relationship between UV and IR exposure accounting for the effects of other factors (28). Because in graphical models if nodes (variables) are not directly connected by a line, then they are conditionally independent, Figure 1C shows that frequency of p53 protein mutation (P-mt) is directly associated with UV and indirectly associated with IR, given UV, P-mt and IR are conditionally independent; i.e. if we were to create a 3 (missense, nonsense, silent) by 2 (high-UV, low-UV) table, the resulting Pearson’s χ²-test applied to it would be significant; however, a 3 (missense, nonsense, silent) by 2 (high-IR, low-IR) table among the low-UV samples and a similar table among the high-UV samples would result in χ² values that would not be significant. The same can be said for the relationship between P-mt and T-ex (time since IR exposure). Simply, Figure 1C illustrates that the difference in frequency of missense and silent p53 mutations out of the total number of mutations as a result of UV exposure, illustrated in Figure 1A, is statistically significant.

The overall frequency of tumors with p53 gene mutations that led to amino acid sequence changes was 70.2% (33/47). Although the frequency of tumors with protein-changing mutations did vary somewhat among the groups, such that there were 69.2% (9/13) in the low-UV/low-IR, 83.3% (5/6) in the low-UV/high-IR, 64.3% (9/14) in the high-UV/low-IR and 71.4% (10/14) in the high-UV/high-IR groups, none of the differences between the high-UV versus low-UV or IR group, or between any two groups, was significant.

With respect to the effects of IR exposure, the graphical modeling analysis showed that the frequency of p53 sequence-site-specific mutations (F-mt), occurring at PyPy, CpG sites (excluding PyPy sequences) and other sites, was significantly associated with IR exposure and with ATB (Figure 2A). That is, Figure 2A shows that the increase in frequency of mutations at CpG sites in tumors from survivors exposed to high doses of IR are statistically higher than the frequency in tumors from non-exposed survivors as measured in a subset of the BCC’s from areas of the body ordinarily not exposed to the sun (low-UV) and the figure also shows

### Table II. Distribution of types of p53 mutations by IR and UV exposure

|                  | IR exposure (%) | UV exposure (%) |
|------------------|-----------------|-----------------|
|                  | High | Low | High | Low |
| Tumors w/any mutation | 17/20 (85.0) | 19/27 (70.4) | 20/28 (71.4) | 14/19 (73.7) |
| Total number of mutations | 44 (100) | 54 (100) | 61 (100) | 37 (100) |
| Mutation type | Missense | 63.6 | 63.0 | 54.1 | 78.4 |
|                | Nonsense | 13.6 | 5.5 | 11.5 | 5.4 |
|                | Silent | 22.7 | 31.5 | 34.4 | 16.2 |
| Mutation site | PyPy | 59.1 | 64.8 | 60.7 | 64.9 |
|                | CpG | 15.9 | 7.4 | 6.6 | 18.9 |
|                | C-to-T at PyPy and CpG | 13.6 | 14.8 | 18.0 | 8.1 |
|                | Other | 11.4 | 13.0 | 14.8 | 8.1 |

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that these mutations are dependent on ATB; it simultaneously shows that UV exposure and F-mt is secondarily associated through IR exposure and ATB. More specifically, the frequency of \( p53 \) mutations at CpG sites (but not at a PyPy sequence) of 38% (5 of 13) in the high-IR group was statistically higher than that of 8% (2 of 24) in the low-IR groups (Figure 2B) among the low-UV group, but this must be interpreted with caution because of multiple comparisons carried out.

There also was a significant relationship between the average number of mutations per tumor and age at exposure. Among those with low-IR exposure, average numbers of mutations, all types and those at CpG sites, increased with increasing age at exposure (Figure 2C, numbers in parentheses). This is likely a reflection of the effect of aging as age at exposure is closely correlated with age at diagnosis. In contrast, it is noteworthy that the average numbers of mutations and the frequency of mutation at CpG and/or PyPy sites per tumor among those with high-IR exposure increased with decreasing age at exposure (Figure 2C).

Finally, LOH analysis of 9q22, the site of \( PTCH1 \) and the site of frequent loss in sporadic BCCs (Figure 3A) revealed a significantly higher deletion rate in the high- than low-IR group (Figure 3B), whereas there was no significant difference in the LOH between the high- and low-UV groups (Figure 3C). Disregarding the IR and UV status, there was a significant increasing trend of frequency of \( PTCH1 \) LOH with decreasing age at exposure to IR (Figure 3D). Deletions in the 9q22 region were determined by LOH analysis using three microsatellite markers in the immediate vicinity of the \( PTCH1 \) gene; this allowed analysis of 32 informative tumors out of the total 47 available. Of the BCC cases we examined 60% had lost heterozygosity at one or other of these sites. Interestingly, there was a statistically significant difference in the association of LOH at microsatellite loci close to the \( PTCH1 \) gene and \( p53 \) mutations; specifically, there were more tumors harboring both \( PTCH1 \) LOH and \( p53 \) mutations than tumors harboring either one or the other or harboring neither in cases exposed to high-IR than to low-IR independent of UV dose (\( \chi^2 = 6.47; P = 0.01 \)) (Table III). However, there was no association when tumors from high-UV and low-UV sites were compared independently of IR dose (\( \chi^2 = 3.35; P = 0.067 \)).

**Discussion**

First, it is noteworthy that the incidence rates of BCC in Japanese are far below those of Caucasians; in the United States, annual incidence rates range from ~180 to 400 cases per 100 000 Caucasians (31,32) and among Caucasian populations in Northern Europe, such as in Germany or Finland, where UV intensity is relatively low, the incidence rates are approximately in the range of 40–50 per 100 000 (33,34) whereas in Japan the rates range from 0.6 to 26 per 100 000 per year (3,35,36). The highest rate of 26 per 100 000 in Japan, found in a population on the southernmost island of Okinawa with relatively high-UV indices, is similar to the rate of 30 per 100 000 in ethnic Japanese living in Hawaii, demonstrating that genetics and exposure to UV radiation play a strong role in basal cell carcinogenesis. Our result, showing an annual incidence rate of ~3 per 100 000, is therefore within the expected range for Japanese. Further,
BCC is among the numerous solid cancers linked to radiation exposure in the studies of the A-bomb survivors. As with several other cancers, the relative risk of BCC related to radiation is increased among those exposed at young ages. The interplay of ionizing radiation with UV in the genesis of human BCC is unknown. Our results showing a significant difference in the number of BCC tumors per person (Table I) in only the high-IR/high-UV versus the low-IR/high-UV groups suggest that the IR effects may be especially pronounced when combined with UV. To characterize the mechanisms of basal cell carcinogenesis, we conducted a molecular analysis of our samples.

There are at least two prominent genes believed to be important in the genesis of BCCs. One is $p53$ and the other is $PTCH1$. Although the $p53$ gene mutation appears to be important in the development of BCC as well as squamous cell carcinoma (SCC) (14,37,38), in the A-bomb survivor population and other IR-exposed populations (39,40), the IR dose response observed for nonmelanoma malignancy appears to be mainly in the form of BCC. These results suggest that other candidate genes, in addition to $p53$ gene, may be involved in BCC development in IR-exposed populations.

LOH at the $PTCH1$ locus has been found in >50% of sporadic BCCs (15,21–23). Inactivating mutations of the remaining allele have been detected in at least 30% of these tumors; most mutations result in truncations of the $PTCH1$ protein (41). It has also been reported that NBCCS patients who receive radiation therapy develop BCC in the radiation field post therapy (42). If the $PTCH1$ gene was a direct target gene of IR, one may expect its deletion to be observed at high frequency in BCC among the highly exposed individuals, and we did find a significantly higher frequency of LOH of the $PTCH1$ locus in BCC among the survivors with high-IR doses compared to those with low doses (Figure 3B). The
lack of a difference in PTCH1 LOH frequencies between the high-UV and low-UV BCC groups may illustrate the different modes of action of ionizing and UV radiations in inactivating the gene. Presumably, analysis of point mutations of the PTCH1 gene, if it had been carried out, would have been able to provide more insights into the possible differences between the high- and low-UV induced tumors. Nevertheless, our results suggest that the PTCH1 gene is one of the key direct target genes critical in the development of BCC following IR exposure.

The analysis of the p53 gene mutations, which are affected by many factors, was more complex and the results were less straightforward, though still noteworthy. The multivariate analysis showed UV to be the primary factor associated with p53 mutation types. More specifically, BCCs in high-UV exposed parts of the body harbored a higher frequency of C-to-T transition p53 mutations at PyPy sequences adjacent to CpG sites—a recognized telltale signature of UV-specific DNA damage (37)—compared to BCCs that occurred in UV-shielded parts of the body UV (Figure 1B). Although the latter finding needs to be treated with caution due to the large number of comparisons made (multivariate), it is in line with biological expectations.

In contrast, LOH and total point mutation frequency of the p53 gene was not IR-dose-dependent. However, we were able to show that p53 mutations at CpG sites at non-PyPy sequences were more frequent in high-IR than in low-IR tumors in UV-shielded parts of the body. This finding also is based on multiple comparisons, but the increasing frequency of sequence site mutations, especially CpG and/or PyPy mutations, with decreasing age at IR exposure is remarkably consistent with the increasing relative risk of BCC with decreasing age at exposure found in the A-bomb survivors (6). It may be that the lack of such a distinction in tumors occurring at heavily UV exposed parts of the body is explained by the possibility that any IR-effects are eclipsed by the characteristic UV-induced C-to-T transitions at PyPy sequences adjacent to CpG sites. Interestingly, the mutations at CpG sites in the p53 gene that were frequently observed in our IR-related BCCs are also often found, albeit at lower frequencies, at these same sites in spontaneous tumors procured from populations of various races (43–45). Most mutations at CpG sites are believed to be caused by deamination of methylated cytosine, and some of them may therefore be the result of attacks by hydroxyl free radicals. Ionizing radiation is known to produce various types of radicals. Wu et al. (46) recently suggested the possibility that radiation-induced reactive oxygen species might generate ‘spontaneous-like’ mutations. CpG sites are also sensitive to breakage by ionizing radiation (47). Therefore, although the frequency of total p53 gene mutations showed no significant increase with exposure dose, the IR dose-dependent increase in p53 CpG mutations and LOH at the PTCH1 locus argues that both kinds of gene alterations are likely to be important in the induction of BCCs by ionizing radiation. Moreover, our data (Table III) also demonstrate that tumors from survivors who were exposed to high doses of IR have a significantly higher frequency of possessing both PTCH1 LOH and p53 mutation than tumors from control populations, independently of UV exposure—implying a possible mechanism for IR-induction of BCC.

The interactive roles of PTCH1 and p53 in cellular responses to IR and to basal cell carcinogenesis are unclear. There are, however, studies that link these two genes; for example, Fujii et al. (48) have reported that cell cycle deregulation via the downregulation of p27 expression becomes apparent following gamma-irradiation of NBCCS cells that possess an intact p53-p21 signaling pathway. In mammalian systems, p27-dependent G1 arrest can be induced by transforming growth factor-β (TGF-β) (49). In Drosophila, sonic hedgehog (SHH), a ligand of PTCH, induces expression of the decapentaplegic (DPP) gene, a homologue of TGF-β(50). Such data provide a possible mechanistic link between irradiation and some cell cycle responses that involve PTCH. A study by Hahn et al. (51) indicates that PTCH heterozygous mice are in excess of four times more sensitive to developmental defects than wild-type mice following exposure to ionizing radiation. These results are even more striking if one considers that p53 heterozygous mice, as well as p53 homozygotes, are also at an increased risk of teratogenesis following ionizing irradiation (52).

Similarly, Aszterbaum et al. (53) and Mancuso et al. (54) showed that chronic-UV and/or acute-IR exposures enhance the growth of BCC in patched heterozygous knockout mice. In these mice, the tumors resemble human BCCs and have Ptch loss of heterozygosity. The UV-induced mouse BCCs had p53 mutations with the predicted UV signature. Moreover, while chronic UV exposure induced SCCs as well as BCCs, acute IR exposure only appeared to induce BCCs. These findings, therefore, mimic both our molecular and previous epidemiological results from human populations.

In fact, there is compelling reason to believe that the process of BCC development is different in people exposed while young and in people exposed while older—evidenced by the higher ERR for BCC development in A-bomb survivors exposed at a young ATB than an older ATB (6). It is logical to assume that the cells of older individuals would have had a larger population of initiated/progressing cells at the time of the bomb than younger individuals, who by definition would have had less time to accumulate spontaneous DNA damage attributable to normal endogenous metabolic activity. On the other hand, the cells of younger individuals may have acquired the critical mutations by IR exposure instead of by long-term endogenous activity. The implication here is of course that those who were older at the time of the bombings may have had a greater probability than the younger ATB population of possessing a pre-existing point mutation affecting one of the PTCH1 alleles or one of another pair of genes important in the early stages of basal cell carcinogenesis (e.g. p53). Our data also demonstrate that there is a statistically significant increasing trend in the frequency of LOH of PTCH1 with decreasing age ATB (Figure 3D), when all cases, high-IR and low-IR, are combined, otherwise, there are insufficient numbers for power. Considering the fact that ionizing radiation is most efficient at creating deletions as a direct action on DNA, our data support the idea that those exposed to the A-bomb at a younger age have a greater tendency to harbor more PTCH1 deletions than those exposed at an older age, who probably harbor more PTCH1 point mutations, which were not analyzed in this study.

Although BCC’s may be less genetically unstable than classically unstable breast cancers, colon cancers and SCC’s, chromosomal instability is nevertheless common in them (55,56). The causes of instability are unknown but the fact
that aberrancy of one gene, PTCH1, is believed to be a major etiological factor of BCC suggests that PTCH1 may promote chromosomal instability. Accordingly, it has been reported that NBCCS-derived cells show innate chromosome instability (57). This suggests that cells with one spontaneously lost or mutated PTCH1 allele (which may be more likely in people who were older ATB) may be prone to losing function of the other allele during the remainder of their life span irrespective of their exposure to IR. Considering that we have shown that exposure to high doses of A-bomb radiation resulted in a significant increase in LOH of markers near the PTCH1 gene, strongly suggesting that the gene itself was a major direct target and was critical in A-bomb-induced BCC genesis, it would then follow that the ensuing increase in genomic instability from the loss of PTCH1 would lead to mutations, in an A-bomb dose-dependent manner, in other genes including those critical in BCC development, such as p53. Our data support this notion of a mutator phenotype by showing that p53 mutations correlate with PTCH1 LOH (Table III); of the high-IR group BCCs, 60% (12/20) have both PTCH1 deletion and p53 mutation compared with only 23% (6/26) of the low-IR group.

This background frequency of simultaneous p53 mutation and PTCH1 LOH is very similar to the 20% (3/15) reported by Kim et al. in an analysis of a Korean BCC population (58) and the 24% (10/42) in a German Caucasian BCC population study by Reifenberger et al. (59) but much less than the 75% (6/8) reported in a Swedish Caucasian BCC population study by Ling et al. (60). Further, the frequency of PTCH1 LOH regardless of p53 mutation in our control group (low-IR) is 38% (10/26) (Hiroshima, lat 34°N) compared with Kim et al.’s 47% (7/15) (Incheon, lat 37°N) (61), Reifenberger et al.’s 48% (20/42) (Dusseldorf, lat 51°N) (59), Ling et al.’s 75% (6/8) (Uppsala, lat 59°N) (60) and Danaee et al.’s 75% (114/151) (New Hampshire, lat 43°N) of US Caucasians (61). Kim et al. (58) and Ling et al. (60) use microdissection techniques to isolate cells for DNA analysis whereas Reifenberger et al. (59), Danaee et al. (61) and we do not, which suggests that the differences in frequencies cannot be completely explained by the method of tumor capture. The incidence rates do not seem to resolve the question; the BCC incidence rates are higher in Finnish than German Caucasians although the latter live at lower latitude and the New Hampshire rate is 6–8 times higher than either of the European rates (62). Genetic factors may also play a role, which could explain the similar frequencies in incidence rates and mutations between the Korean and Japanese populations as well as the dissimilar frequencies among the Caucasian populations. For example, Caucasians of Celtic origin are known to have elevated risks for skin cancers (63). In some aspects, Caucasians in the German study may share certain genetic traits critical in basal cell carcinogenesis with Orientals compared with Caucasians in the Scandinavian countries and the United States (New Hampshire). Interestingly, one study showed that Irish, Danish and Swedish Caucasians were most associated with a Pro/Pro polymorphism within PTCH1 exon 23 at codon 1315 that also correlated with BCC risk and severity compared to a Pro/Leu or Leu/Leu genotype. (64) Further, in cases of PTCH1 LOH, there appeared to be a non-random loss in favor of retention of the Pro allele. Therefore, although the frequencies of the codon 1315 polymorphism (C3944T) in the Japanese population are unknown, our data predict that, because IR is most efficient in producing deletions rather than point mutations, persons who possess the Pro/Leu C3944T polymorphism are at a higher risk of developing BCC following exposure to IR than those with the Leu/Leu.

It would be interesting to analyze this polymorphism in future studies of BCC’s from the A-bomb population.

Finally although the overall p53 mutation frequencies do not statistically correlate with A-bomb dose, our data do show a significant increase in the mean number of p53 mutations, especially at CpG and/or PyPy sequences, per tumor with decrease in ATB among survivors exposed to high-IR doses (Figure 2C). As it is likely that tumors contain multiple clones with different p53 mutations (65,66), this observed trend in mutation frequency per tumor could be interpreted as an increase in tumor heterogeneity with younger ATB.

The reasons for such an observation are unclear but we present one possible explanation. It is believed that p53 dysfunction is a critical early step, soon after PTCH1 dysfunction, in basal cell carcinogenesis. Thus all wild-type cells, found in greater numbers in younger people, exposed to A-bomb radiation and incurring a PTCH1 mutation that in turn causes a p53 mutation, through induction of genomic instability, have the potential of progressing toward BCC; whereas most pre-BCC cells, found in greater numbers in older people, will already have harbored the necessary PTCH1 and p53 mutations at the time of the bombing and thus any p53 mutations that could have been induced by the A-bomb will not necessarily be selected and clonally expanded as the cells progress toward BCC. In other words, it is more likely that the early lesions necessary for the development of BCCs in the young-ATB are attributable to the A-bomb radiation than early lesions in the origins of BCCs arising in the old-ATB, which may help to mechanistically explain our current results and in turn molecularly explain the epidemiological data for BCC development that show an ERR (for >1 Gy-exposed survivors vs. 0-5 mGy-exposed survivors) for those who were young-ATB is significantly greater than it is for those who were older-ATB (6).

Accordingly, in the population who were young at exposure, it may be that ionizing radiation directly induced the loss of at least one of the alleles of the PTCH1 gene. A dysfunctional PTCH1 could have then induced a mutator phenotype and led to mutations in the p53 gene; this is supported by our data showing lack of correlation between A-bomb dose and deletion of p53 but a correlation with types of p53 mutations generally attributed to endogenous metabolic activity. A molecular study of hepatocellular carcinomas in A-bomb survivors also suggests a mutator phenotype induction of p53 mutations (25). Over the span of decades, such cells, ‘primed’ for basal cell carcinogenesis, may well have accumulated other mutations. Those mutations, if in critical genes, could have conferred further survival advantage, thus spurring the progression of the cell along the path to BCC. It would be interesting to analyze the remaining PTCH1 allele in future studies to test whether this gene resembles p53 in also having acquired multiple point mutations.

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