



Comparison of *TP53* mutation analysis findings for tar pitch dermatopathy and ionizing radiation-related skin cancer

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Background: Tar has been well known as a carcinogen ever since Yamagiwa artificially induced carcinogenesis for the first time by painting a rabbit ear with tar 100 years ago. While humans can be exposed to tar under numerous circumstances, few molecular pathological analyses of skin lesions caused by “tar pitch dermatopathy” have been performed because tar pitch dermatopathy itself is currently rare.

Methods: A mutation analysis of *TP53* was conducted using long-term stored formalin-fixed, paraffin-embedded tissue specimens of tar pitch dermatopathy in 4 subjects; a similar analysis of radiation-induced skin cancer in 2 subjects was also performed for comparison. Multiple sites were analyzed.

Results: Among the specimens from the 4 subjects with tar pitch dermatopathy, the mutations c.625A>T and c.715A>G were each found in 1 of the lesion specimens, whereas the wild type for *TP53* was seen in all the non-tumorous skin samples from these cases. The additional mutation c.503A>C was also detected in a different portion of the specimen from the case in which c.715A>G had been detected. In contrast, in 1 of the 2 cases with radiation-induced skin cancer (3 squamous cell carcinoma lesions), the novel mutations c.224delC and c.470delT were found at dipyrimidine sites of *TP53*. Thus, the mutation spectrum found in tar pitch dermatopathy did not include G>T transversions arising from benzo(a)pyrene, as expected, but instead included the mutation c.625A>T, which is prevalent among tobacco-related cancers according to the IARC database.

Conclusions: Skin malignancy developing under peculiar conditions provides an opportunity to investigate the human reality of environmental carcinogenesis. Although a small piece of data this information will be helpful for understanding human skin carcinogenesis in terms of occupational exposure to environmental substances and informative in precision medicine especially in precision prevention.

Keywords: *TP53*; mutation spectrum; Tar-pitch dermatopathy; skin cancer; radiation; environmental carcinogenesis

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Introduction

Tar played an important role in the first successful artificial induction of carcinogenesis in rabbits by Yamagiwa *et al.* (1) and has been identified as an occupational risk factor for various skin diseases since. Among them, tar pitch dermatopathy is mainly of historical importance, and it encompasses both early lesions, such as pigmentation and acne, and late lesions, including acanthoma, Bowen's disease, and squamous cell carcinoma (2). By the middle of the 20th century, half of all industrial skin cancers were attributed to exposure to tar and pitch in occupations such as coal mining and engineering (3). Coal tar has also been used as a therapeutic regimen for psoriasis as represented by Goeckerman therapy, a combination therapy consisting of crude coal tar and ultraviolet B radiation (4). However, tar pitch dermatopathy itself is very rarely encountered in clinical practice at present because of ecological regulations and occupational health control, and almost no molecular analyses of this type of skin lesion have been performed. On the other hand, x-ray exposure is another historical inducer of occupational skin cancers, typically squamous cell carcinoma (2) occurring in the hands and fingers of radiology professionals. CC to TT tandem transitions and C to T transitions at dipyrimidine sites are well-known mutation signatures for these types of lesions (5).

Even nowadays incidental exposure to environmental or industrial carcinogens brings about a cluster of peculiar cancers and DNA fingerprints (or mutation signature) of these cancer tissues provide our understanding of the reality and novelty of human carcinogenesis (6). Tar-pitch dermatopathy is rare and will be rarer entity, but the findings here, though anecdotal will add some piece of insights on accidental human carcinogenesis.

Methods

We examined the *TP53* status in cutaneous lesions related to tar pitch dermatopathy in 4 Japanese patients. Multiple sites including non-tumorous portions were taken from each lesion (Figure S1) and examined. These 4 cases had known occupational exposure to coal tar. For comparison, the same procedures were performed for skin cancers that had occurred on the fingers of 2 patients (3 lesions) with occupational exposure to radiation. The occupational records and diagnoses are shown in the Table 1.

Using samples stored in pathology archives, DNA was extracted from multiple sites of each lesion. Macroscopically

and manually multiple portions were taken and DNA analysis were conducted (Figure S1). PCR amplification was not successful for some of the DNA samples from these multiple sites in an individual case. Finally, confirmative sequences could be obtained in 8 lesions in 4 cases. For a sample in which PCR amplification was successful, ordinary Sanger sequencing was performed according to previously described methods (7). Primers covering exons 4 to 8 of *TP53* were used and are available upon request. The entire procedure was repeated more than twice to confirm the mutation findings.

An immunohistological study was performed using a standard method, and the clone DO-7 (Dako Japan, Tokyo) was used to reveal the distribution of p53 protein in the tissues (8). This DO-7 antibody identifies both the wild type and the mutated form of the p53 protein and some mutant p53s has longer half-life in the cells. Wider varieties of p53 are known currently but the majority of missense mutants are detectable by this antibody and DO-7 is widely used in practice, too (9).

The numbers of the cases and clones were few and statistical interpretation was not done.

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by Institutional Review Board of Hamamatsu University School of Medicine (No. 20-011). Informed consents were not required from the participants, because the study was conducted by using residual pathological archives in anonymous way and no intervention was included in any part of the study.

Results

Base substitution-type mutations (missense and nonsense types) were found in skin cancer arising from tar pitch dermatopathy. The histopathology of the cases and sequence graphs are shown in Figure 1. In case 1 (Figure 1A) and case 2 (Figure 1B) both exhibit squamous cell carcinoma in dermatopathy. Representative sequenograms are shown in Figure 1C-1G. While the mutation c.715A>G was identified (Figure 1C) in one portion, and an additional c.503A>C mutation (Figure 1E) was found in another portion (double mutation in this portion), no mutation was found in case 2 though almost similar histology. In case 4, the mutation c.625A>T was identified (Figure 1D). This mutation generates a stop codon, and the cancer specimen was negative for immunoreactivity to p53 protein (data not shown). In contrast, different deletion-type mutations

Table 1 Patient characteristics

Case	Age at diagnosis/ sex	Occupation [location of lesion(s)]	Duration of exposure (years)	Pathology	<i>TP53</i> mutation (exons 4 to 8)	Outcome
1	Late 70s/M	Fisherman (multiple sites: foot, leg, and arm)	50	Squamous cell carcinoma	c.[715A>G(;);503A>C]; p.[(N239D(;);H168P)]	Unknown
2	50/M	Fisherman (buttock)	47	Squamous cell carcinoma	None	Unknown
3	61/M	Iron Foundry (left knee)	39	Bowen's disease	None	No recurrence
4 [#]	77/M	Iron Foundry (left hand)	30	Squamous cell carcinoma	c.625A>T; p.(R209*) (note)	Death due to metastasis
5	79/M	Radiology engineer (finger)	22	Squamous cell carcinoma	c. [224delC(;); 470delT]; p.[(P75Lfs*48(;);V157Afs*13)] (codon 75 CCT>CT) (codon 157 GTC>GC)	Alive
6	47/M	Orthopedic surgeon (finger)	25	Squamous cell carcinoma	None	Alive

[#], this mutation has also been found in a small cell lung cancer cell line (H2141). M, male.

(Figure 1F,1G) were found at different sites in one of the two cases with occupational radiation exposure. Figure 1H is p53 protein immunostaining in the case 1. Nearly clonal p53 protein staining is noticed.

Discussion

Even in the era of massive parallel sequencing, *TP53* is regarded as a gene that very frequently exhibits somatic mutations in most common tumors; thus, the mutation spectrum of *TP53* can provide etiological clues regarding human carcinogenesis (10). Tar contains many carcinogens, with benzo[a]pyrene being one of the most potent and extensively investigated substances. Benzo[a]pyrene is metabolized to benzo[a]pyrene-7,8-diol-9,10-epoxide (BPDE), which binds to guanine covalently and induces mispairing and mutation to thymine at that guanine position (11). This pathway agrees with mutation spectrum findings in human tobacco-associated lung cancer (12). Unexpectedly, the coal tar-related skin cancers examined in this study did not harbor any typical G to T transversions in *TP53* within the region spanning exons 4 to 8, though multiple sites were examined. The reason for this unexpected finding is unknown.

On the other hand, the somatic c.625A>T (p.R209*) mutation, found in the present study, has been recorded in 18 cases in the IARC database (13). Interestingly, 15 of these cases were cancers that were strongly correlated

with tobacco smoking (lung, esophagus, urinary tract, hypopharynx, and oropharynx). The other three tumors were soft tissue leiomyosarcoma, breast, and ovarian cancer. A germline mutation of this type has also been recorded. None of the presently reported cases had a family history of cancer, and wild-type *TP53* was detected in non-tumorous skin samples.

Fifty-two cases of somatic c.715A>G mutations have been included in the IARC database, 19 of which were tobacco-related cancers. Meanwhile, the c.503A>C mutation has been identified in 14 entries, 4 of which were tobacco-related cancers. No skin cancers with the mutations found in the present analysis have been included in the IARC *TP53* database (R20 release) (13). The presently reported mutation type suggests that these mutations are caused by environmental factors other than sunlight (the commonest cause of skin cancer), such as tar-pitch-related oxidative damage including adenine deamination (14).

In skin cancer, which is most commonly related to UV or sunlight exposure, the mutation spectrum favors CC to TT changes via the formation of pyrimidine dimers and C to T transitions at dipyrimidine sites (15). Actually, as shown for comparison, the two novel mutations found in one of our cases with a strong background of occupational ionizing radiation occurred at CC and CT positions (codon 75 was CCT, codon 157 was CTG, case 5), as would be generally expected, and our hypothesis seems to be reasonable.

Takata *et al.* (16) found 9 mutations in Japanese basal cell

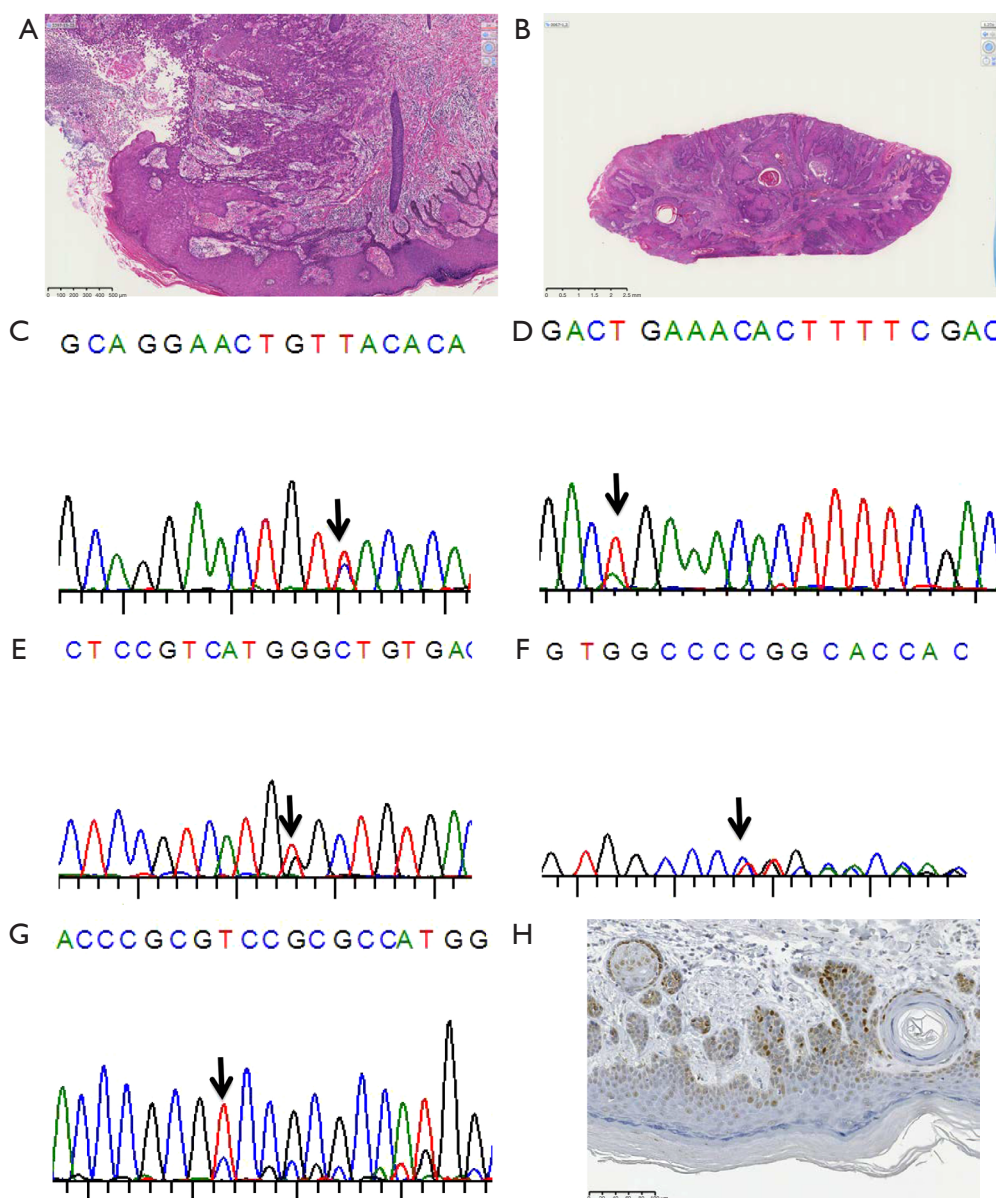


Figure 1 The histological and immunohistological view and sequence results of the representative cases. (A) Case 1: squamous cell carcinoma (HE staining); (B) case 2: squamous cell carcinoma (HE staining); (C) case 1: c.715A>G (shown in reverse direction); (D) case 4: c.625A>T; (E) case 1: c.503A>G (shown in reverse direction); (F) case 5: c.470delT; (G) case 5: c.224delC; (H) case 1: squamous cell carcinoma, p53 immunostaining.

carcinoma, 2 of which were G to T transversions, but no mutations from adenine were listed. They did not mention any particular exposure history for these subjects. We cannot draw any definitive conclusions based on the small number of cases in the present study, but the situation in tar pitch dermatopathy appears to be quite different from that in other skin cancers and to be more similar to that

occurring in tobacco-related cancer, though typical benzo[a]pyrene-induced mutations were not identified. In a recent study, anti-BPDE-DNA adducts were measured in psoriasis patients who had received Goeckerman therapy (17), but straightforward correlations between the antibody level and the exposure attributes were not found. Thus, the effect of tar-pitch is not always and not simply that of benzo[a]

pyrene.

This study has inevitable limitations. The numbers of the cases are small and anecdotal. The samples are old archives, thus, extensive sequence analysis using next generation sequencing was not possible. We did make only manual dissection to isolate epidermal tissue with and without malignant or premalignant changes, thus single cell level analysis which combined immunohistochemical detection of p53 protein with sequence results was not done.

Very recently, massive parallel sequencing has identified somatic mutations in normal-looking skin; thus, our observations reported here may represent a final phase in the clonal evolution of squamous cell carcinoma (18). Although multiple sites were analyzed, PCR was not always successful, probably because the fixation and storage condition were uncontrollable. Further elucidation of the steps in clonal evolution in these particular settings is needed, and a molecular signature study of skin cancers with specific occupational incidences (when not avoidable) may provide a clue to an improved understanding of human skin carcinogenesis. Tar-Pitch dermatopathy and other accidental occurrences of human cancer may happen someday. Even for such an incidence careful preservation and preparation of specimens for future research is necessary.

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Footnote

Data Sharing Statement: Available at <https://jlp.amegroups.com/article/view/10.21037/jlp-21-69/dss>

Peer Review File: Available at <https://jlp.amegroups.com/article/view/10.21037/jlp-21-69/prf>

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September 2023. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by Institutional Review Board of Hamamatsu University School of Medicine (No. 20-011). Informed consents were not required from the participants, because the study was conducted by using residual pathological archives in anonymous way and no intervention was included in any part of the study.

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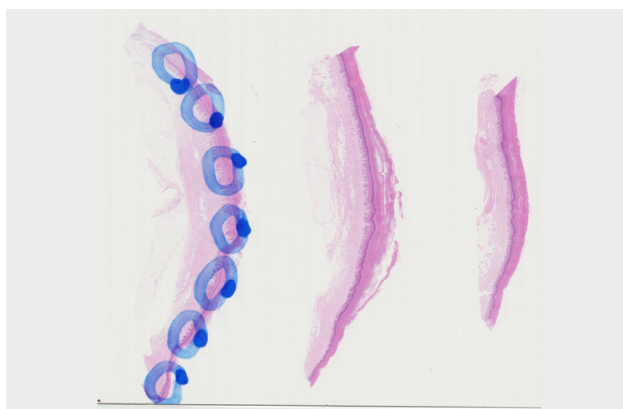


Figure S1 All the specimen were deposited as virtual slides before making punctures (blue circles) for extraction of DNA from these cylinders. Multiple sites were taken and tried to extract DNAs from all of the sites, but PCR-sequencing was successful only in some of them, thus the further information such as field cancerization view was not available.