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Squamous Cell Carcinomas in Patients with Fanconi Anemia and Dyskeratosis Congenita: A Search for Human Papillomavirus

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Abstract

Patients with Fanconi anemia (FA) and dyskeratosis congenita (DC) are at high risk of head and neck and anogenital squamous cell carcinomas (SCC). In the general population, these sites (particularly oropharyngeal SCC) may be associated with infection with human papillomavirus (HPV). In FA and DC, however, the majority of HNSCC occur in the oral cavity. We investigated the HPV status of HNSCC and vulvar SCC from 9 patients with FA and 4 with DC using a very sensitive PCR assay, and found HPV16 DNA in only a single vulvar tumor from one FA patient, and in none of the HNSCC. These results suggest that HPV may not be the cause of SCC in patients with FA or DC, and that vaccination may not reduce the incidence of HNSCC in these patients.

Keywords

Fanconi anemia; dyskeratosis congenita; squamous cell carcinoma; human papillomavirus

Fanconi anemia (FA) is primarily an autosomal recessive DNA repair disorder characterized in many patients by congenital abnormalities, bone marrow failure, acute myeloid leukemia, and solid tumors. The relative risk of tumors is ~50-fold higher than in the general population, ~800-fold for head and neck squamous cell carcinomas (HNSCC) and ~3000-fold for vulvar cancer. Dyskeratosis congenita (DC) is a clinically similar inherited bone marrow failure syndrome, due to abnormalities in telomere biology, with a relative risk of ~8-fold for solid tumors, and ~1100-fold for HNSCC (1).

In the general population, almost all cervical, and up to half of vulvar, cancers are associated with human papillomavirus (HPV) infection, particularly the high-risk types HPV16 and HPV18 (2). In the U.S., HPV16 or 18 is also associated with ~25% of HNSCC, particularly >50% of oropharynx rather than oral cavity (<10%) (3–5). A possible common feature relating the specific gynecologic and head and neck sites is a tissue transformation zone (6). Gillison *et al.* indicated that a prophylactic vaccine has potential to prevent cervical as well

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There have been two contradictory reports with regard to the role of human papillomavirus (HPV) in head and neck and gynecologic squamous cell carcinoma (SCC) in patients with Fanconi anemia. We studied tumors from patients with Fanconi anemia or dyskeratosis congenita, and did not find evidence for causality by HPV. We predict that vaccination with current HPV vaccines may not reduce the incidence of SCC in these patients.

as specific non-cervical cancers, including vulvar, vaginal, anal, penile and oropharyngeal, with weaker evidence for prevention of cancers of the oral cavity, larynx, and esophagus (7).

Data on the role of HPV in cancer in FA are limited, and there are no studies in DC. Kutler *et al.* reported HPV DNA (90% HPV16) in 84% of 25 tumors from 24 FA patients, including 6 vulvar and 15 oral cavity (8). However, positive tumors had as little as 1 copy of HPV DNA per 10 cells; this measure may have been too sensitive, leading to an excess of clinical false positive results. Van Zeeburg *et al.* found HPV16 in only 2 of 3 anogenital SCCs and none of 16 HNSCC or 2 esophageal SCCs; positives were identified by the broad spectrum HPV GP5+/6+ PCR enzyme immune assay and quantitative PCR (9). These discrepant studies differ in geography (USA *versus* Europe), methods, and assay sensitivities, and did not study additional markers to distinguish transient vs transforming HPV infections.

The association of HPV with cancer in FA is plausible, given the locations of the cancers. However, vulvar cancer is much more common than cervical cancer in FA (unlike the general population in which the reverse pertains), and oral cavity cancer is more common than oropharyngeal. In a review of 2250 FA cases in the literature (10) we found 26 patients with gynecologic cancers among nearly 1000 females (not age-adjusted): 7 were in the cervix and 24 in the vulva; 5 involved both sites. Among 113 patients with HNSCC, 31 arose in the oropharynx, 65 in the oral cavity, and in 18 the site was not specified. Five women with gynecologic cancer also had an independent primary oral cavity SCC. Our experience with the National Cancer Institute (NCI) FA cohort is similar: 1 of 6 gynecologic SCC was cervical, while 5 were vulvar; 5 of 14 HNSCC arose in the oropharynx *versus* 9 in the oral cavity (1). Hence, only 20% of gynecologic cancers and 30% of HNSCC in FA patients in the literature and in our NCI cohort were in the sites at which HPV is more likely to be identified in the general population.

The findings in DC patients were similar. Twenty-three of more than 650 cases in the literature had HNSCC: 20 in the oral cavity, 4 in the oropharynx, (2 in both), and 1 was not stated; one DC subject had cervical cancer. In our NCI cohort, 5 DC patients had HNSCC (2 oropharynx, 3 oral cavity), and one had cervical SCC (1).

Because of the scanty and contradictory findings in the literature, we examined HPV in tumor specimens from 5 HNSCC (2 male, 3 female) and 4 vulvar/vaginal SCC from a total of 9 FA patients, as well as HNSCC from 4 patients (1 male, 3 female) with DC (Table 1). DNA was extracted from paraffin-embedded tumor samples which were confirmed to contain SCC. HPV was sought using PCR with SPF-10 broad-spectrum primers targeting the L1 ORF, followed by selection of HPV positive samples by DNA enzyme immune assay (DEIA), which can detect 54 different anogenital HPV types (11). Amplimers from HPV positive samples were genotyped for 25 common anogenital HPV types with the LiPA₂₅ method (12). Because of the small amplification product of this primer set (65 base pairs), the method is well-suited for fixed tissue, where there might be partial degradation of DNA. HPV16 DNA was identified in only one sample, a vulvar tumor from a 24 year-old FA patient. P16^{INK4a} stained diffusely and strongly indicating active E7 oncogene expression (12). In the combination of all tumors, FA and DC, HNSCC and gynecologic, we found that only 1 in 13 was positive, significantly different from the 84% reported by Kutler ($p < 0.001$). Restriction to FA alone provided 1 positive out of 9, also significantly different from the Kutler result (8). In order to confirm our results, the samples were also analyzed with a multiplex type specific PCR targeting the E6 ORF (13). No additional HPV positive samples were found.

Our results resemble those of van Zeeburg *et al.*, in which HPV does not appear to be associated with HNSCC (9), suggesting that HPV may not be the only cause of HNSCC or gynecological cancer in patients with FA or DC. Our conclusion is limited by the small number of tumors, but re-opens the question about the role (if any) played by HPV in SCC in patients with mutations in genes in the FA/BRCA DNA repair pathway or in telomere biology. Only 1 of the DC patients with tongue cancer reported any smoking, while 5 of the 9 patients with HNSCC reported light drinking. The possible pathways for the SCC are subject to speculation, and presumably involve potential interaction between factors relevant in the general population and mutations in the FA/BRCA DNA repair pathway, or the telomere biology pathway.

The availability of 2 prophylactic HPV vaccines is very enticing for their theoretical potential to prevent SCC in those disorders, but our data and those of van Zeeburg *et al.* suggest that HPV immunization is not likely to reduce the incidence of SCC in either FA or DC. Nonetheless, we favor immunizing FA and DC patients according to general guidelines (all individuals between ages 9 and 26). Continued longitudinal follow-up of immunized FA and DC patients will determine whether their risk of SCC declines. However, since these cancers generally develop in young adults, and the number of FA and DC patients is very small, it will take many years to collect this information. In the meanwhile, our limited data plus those previously reported by van Zeeburg *et al.* suggest that we must focus our etiologic research on mechanisms unrelated to HPV for SCC in these patients.

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Table 1

HPV Status of SCC from Patients with FA or DC

Syndrome	UPN	Age, yrs	Sex	Tumor	HPV type
Fanconi anemia	1-1	35	M	Tongue	-
	7-1	47	F	Gingiva	-
	9-1	42	F	Tongue lateral	-
	33-1	31	F	Oropharynx	-
	289-1	52	M	Tongue base	-
	5-1	31	F	Vagina	-
	144-1	32	F	Vulva	-
	162-1	24	F	Vulva	16
	169-1	25	F	Vulva	-
Dyskeratosis congenita	3-1	25	M	Tongue (base)	-
	114-1	50	F	Tongue lateral	-
	145-1	14	F	Lip	-
	226-2	28	F	Tongue lateral	-

Abbreviations: UPN, unique patient number; M, male, F, female, no HPV was detected. 16, only HPV16 was detected.