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Papillomaviral DNA and increased p16^{CDKN2A} protein are frequently present within feline cutaneous squamous cell carcinomas in ultraviolet-protected skin

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Abstract

Squamous cell carcinomas (SCCs) are common feline skin tumours. While exposure to ultraviolet (UV) light causes some SCCs, a subset develop in UV-protected skin. In cats, papillomaviruses (PVs) cause viral plaques and Bowenoid *in situ* carcinomas (BISCs). As both may progress to SCC, it was hypothesized that SCCs in UV-protected skin may represent neoplastic transformation of a PV-induced lesion. To investigate this hypothesis, PCR was used to amplify PV DNA from 25 UV-protected and 45 UV-exposed SCCs. Oncogenic human PVs cause neoplasia by mechanisms that also increase p16^{CDKN2A} protein (p16). As increased p16 is present in feline viral plaques and BISCs, immunohistochemistry was used to detect p16 within the SCCs. Papillomaviral DNA was amplified from 76% of UV-protected SCCs, but only 42% of UV-exposed SCCs. Increased p16 was present in 84% of UV-protected SCCs, but only 40% of UV-exposed SCCs. The more frequent detection of PV DNA and increased p16 within UV-protected SCCs supports the hypothesis that some develop from a PV-induced plaque or BISC. *Felis domesticus* PV-2 is thought to cause viral plaques and BISCs. This PV was detected most frequently within the UV-protected SCCs, supporting development from a PV-induced lesion. Increased p16 and PV DNA were less frequent within UV-exposed SCCs, presumably because these developed from actinic keratosis rather than a PV-induced lesion. The results support the hypothesis that some feline cutaneous SCCs are caused by PV infection and suggest that PVs may cause neoplasia by mechanisms that also increase p16.

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Introduction

Squamous cell carcinomas (SCCs) are the most common malignant skin neoplasm of cats and can be subdivided into those that develop in areas that are exposed to ultraviolet (UV) light and those that are not. Areas with high UV exposure include the lightly pigmented, poorly haired skin of the pinna, nasal planum and eyelids. Most SCCs that develop in UV-exposed skin are thought to develop as a progression of actinic keratosis and are caused by chronic UV exposure.[1] In contrast, SCCs that develop in densely haired, pigmented skin are protected from UV light and are unlikely to be caused by chronic UV exposure. Papillomaviruses (PVs) have been associated with skin cancer in many species.[2] In cats, PVs are thought to cause feline viral plaques and Bowenoid *in situ* carcinomas (BISCs).[3,4] As both viral plaques and BISCs have been reported to progress to SCCs,[1,5] it was hypothesized that feline SCCs in UV-protected skin can develop from a viral plaque or BISC. If feline SCCs in UV-protected skin do develop as a progression of a PV-induced lesion, this would suggest that a proportion of feline SCCs are caused by PV infection.

Papillomaviral DNA is detectible within 18–85% of feline cutaneous SCCs.[3,6] However, PV DNA is frequently detectible in clinically normal feline skin.[7] Therefore, when a PV is detected within a SCC it is difficult to determine whether the PV caused the neoplasm or is an 'innocent bystander'. In the present study, the rate of detection of PV DNA within SCCs from UV-protected skin was compared with the rate within SCCs from UV-exposed skin. As SCCs in UV-exposed skin develop from actinic keratosis, they are less likely to be caused by PV infection, and PVs detected within these SCCs may be 'innocent bystanders'. However, if SCCs in UV-protected skin more frequently contain PV DNA than UV-exposed SCCs, this would support a role for PVs in the development of these SCCs. Around 25% of human oral SCCs are caused by PV infection.[8] Oncogenic human PVs consistently increase p16^{CDKN2A} protein (p16), and immunohistochemistry to detect p16 can be used to determine whether a human oral SCC was caused by PV infection.[9] In cats, neither increased p16 nor PV DNA were detected within 30 oral SCCs.[10] However, increased p16 and PV DNA were consistently detected in a series of feline PV-induced skin lesions.[11] Therefore, if

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increased p16 is more frequently present within feline SCCs in UV-protected skin than in UV-exposed SCCs, this would further support a role of PVs in the development of feline SCCs in UV-protected skin.

Materials and methods

Squamous cell carcinomas were identified by retrospective surveys of databases at Massey University, New Zealand Veterinary Pathology Ltd and Gribbles Veterinary Pathology Ltd. Cases were only included when the location of the SCC was reported within the submission. The fixation conditions of the neoplasms were unknown. The diagnosis was confirmed using histological examination of a haematoxylin and eosin stained section. For the purpose of the study, locations on the body were subdivided into UV-exposed if the area contained little protective hair, while densely haired areas were considered UV-protected. Therefore, SCCs on the pinna, nasal planum, eyelid or third eyelid were considered to be UV exposed, while UV-protected SCCs were from the face, digit, thigh or neck.

Sections for immunohistochemistry were deparaffinized in xylene, rehydrated in graded ethanol and rinsed in distilled water. Immunohistochemistry was performed using a mouse anti-human p16 monoclonal antibody (BD Biosciences, San Jose, CA, USA) as previously described.[11] The percentage of cells demonstrating intense nuclear and cytoplasmic immunoreactivity was estimated by examining five different ×400 fields within each lesion. Squamous cell carcinomas were considered to contain increased p16 if >90% of the nuclei or cytoplasm of the neoplastic cells contained intense p16 immunoreactivity. Epithelial cells within the basal layer often exhibited weak immunoreactivity and were used as an internal positive control. Superficial layers of the surrounding epidermis were used as a negative control. All immunohistochemical evaluation was performed by a single investigator (J.S.M.) who was unaware of the area from which the SCC had been removed. The specificity of anti-human p16 antibodies for feline p16 has been previously documented.[12]

DNA was extracted from formalin-fixed, paraffin-embedded samples as previously described.[13] Four primer sets were used to amplify PV DNA from the SCCs. These included the JMPF/R primer set, which specifically amplifies *Felis domesticus* papillomavirus-2 (FdPV-2) DNA[3] and the MY09/11 consensus primers that amplify PVs from multiple species.[14] The consensus primers amplified two PV DNA sequences and the JMY2F (5′-TCA GGG CAC GCA AGA ACT GCA-3′), JMY2R (5′-ACC TGT CTG TGG TGC AGG AAC A-3′) and JMY3F (5′-GGT CAG TAT AAC CCA GAG CAG AGC A-3′), JMY3R (5′-AGG AGG CAC CTT GTC TGG GCA-3′) primers were designed to specifically amplify these. Final concentrations of the reaction products using the JMY2F/R and JMY3F/R primer sets were 1× PCR buffer, 1.5 mM $MgCl_2$, 200 μM dNTP, 0.25 μM of each primer, 1.25 units DNA polymerase and 2.5 μL template DNA in a final reaction volume of 30 μL. Amplification conditions were 94 °C for 10 min, followed by 45 cycles of 94 °C for 1.5 min, 60 °C for 1.5 min and 72 °C for 1.5 min. The final extension was at 72 °C for 5 min. Electrophoresis in a 1% agarose gel containing ethidium bromide was used to detect the 202 bp fragment using the JMY2F/R primers and the 246 bp fragment amplified by the JMY3F/R primers. All PCRs were carried out in duplicate. DNA extracted from a feline viral plaque and a bovine fibropapilloma was used as a positive control for the JMPF/R and MY09/11 primers, respectively. DNA extracted from a bovine fibropapilloma was used as a negative control for the JMPF/R, JMY2F/R and JMY3F/R primers, while the negative control for the MY09/11 primers did not contain template DNA. When no PV DNA was amplified from a sample, the presence of amplifiable DNA within the sample was confirmed by amplifying part of the feline p53 gene as previously described.[15]

Due to the previously demonstrated specificity of the JMPF/R primers for FdPV-2,[3] DNA amplified by these primers was not sequenced. However, DNA amplified by the consensus, JMY2F/R and JMY3F/R primers was purified and sequenced as previously described.[13] Results were compared with known sequences in GenBank (http://www.ncbi.nlm.nih.gov/genbank) using the basic local alignment search tool (http://www.ncbi.nlm.nih.gov/blast). Sequences were compared with each other using Geneious 2.0.10 software (Biomatters Ltd, Auckland, New Zealand). Differences between groups were investigated by analysis of variance using SPSS version 16 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Seventy feline SCCs were identified. Forty-five were from UV-exposed skin, including 16 from the nasal planum, 14 from the pinna, 10 from the eyelids and five from the third eyelids (Table 1). Twenty-five SCCs were from UV-protected skin, including 13 from the face, six from the digit, four from the thigh and two from the neck. The ages of 60 affected cats were known. The mean age of the cats with both UV-exposed and UV-protected SCCs was 11.2 years.

Immunoreactivity against p16 was remarkably biphasic, with the SCCs either containing intense immunoreactivity in almost all the neoplastic cells or weak immunoreactivity within only a small proportion. Increased p16 was visible within 39 of 70 (56%) SCCs (Figures 1 and 2), while 31 of 70 (44%) SCCs contained only weak nuclear or cytoplasmic immunoreactivity within <20% of the neoplastic cells (Figure 3). Increased p16 was more frequent in SCCs from UV-protected skin than in UV-exposed SCCs ($P < 0.001$). Within the SCCs from UV-exposed skin, increased p16 was more frequent in SCCs from the nasal planum than in SCCs from the pinna ($P = 0.02$). There was no significant difference in the age of cats with SCCs containing increased p16 and cats with SCCs without increased p16 ($P = 0.35$).

Papillomaviral DNA was amplified from 38 of 70 (54%) SCCs. Squamous cell carcinomas from UV-protected skin more frequently contained PV DNA than SCCs from UV-exposed skin ($P = 0.006$). Papillomaviral DNA was amplified significantly more frequently from SCCs with increased p16 than from SCCs without increased p16

Table 1. The presence of cellular $p16^{CDKN2A}$ protein (p16) and papillomaviral DNA within 70 feline cutaneous squamous cell carcinomas

Location of neoplasm	P16+	PV+
All locations	39 (56%)	38 (54%)
UV-exposed skin	18 (40%)	19 (42%)
Nasal planum	9 (56%)	9 (56%)*
Pinna	2 (14%)	5 (36%)
Eyelid	5 (50%)	4 (40%)†
Third eyelid	2 (40%)	1 (20%)
UV-protected skin	21 (84%)	19 (76%)
Face	11 (85%)	10 (77%)
Digit	6 (100%)	5 (83%)
Thigh	2 (50%)	2 (50%)‡
Neck	2 (100%)	2 (100%)

Abbreviations: P16+, >90% of the neoplastic cells contain intense p16 immunoreactivity; PV+, papillomaviral DNA was amplified from the neoplasm; UV, ultraviolet light. *Only DNA sequences of FdPV-2 were detected in three SCCs, only FdPV-MY3 in two, FdPV-MY2 and FdPV-2 in two, FdPV-MY3 and FdPV-2 in one, and only FdPV-MY2 in one. †Only DNA sequences of FdPV-2 were detected in three SCCs, and only FdPV-MY2 in one. ‡Only DNA sequences of FdPV-2 were detected in one SCC and only FdPV-MY2 in one.

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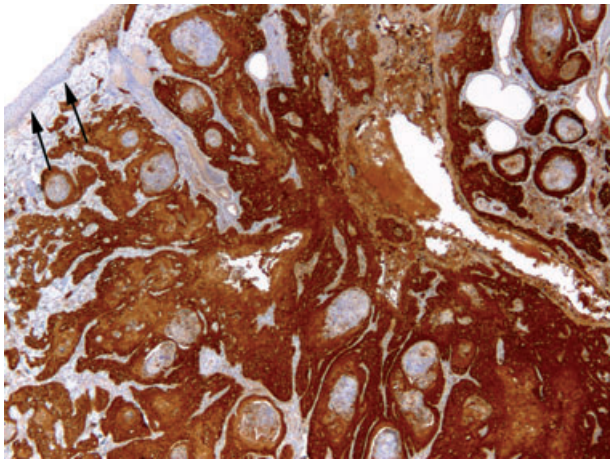

Figure 1. Photomicrograph of a feline squamous cell carcinoma removed from the pinna. Note consistent, intense p16 immunoreactivity within the neoplastic cells. Non-neoplastic epidermis is also visible within the photomicrograph (arrows). Papillomaviral DNA was amplified from this neoplasm. 3,3′-Diaminobenzidine substrate with haematoxylin counterstain. Magnification ×50.

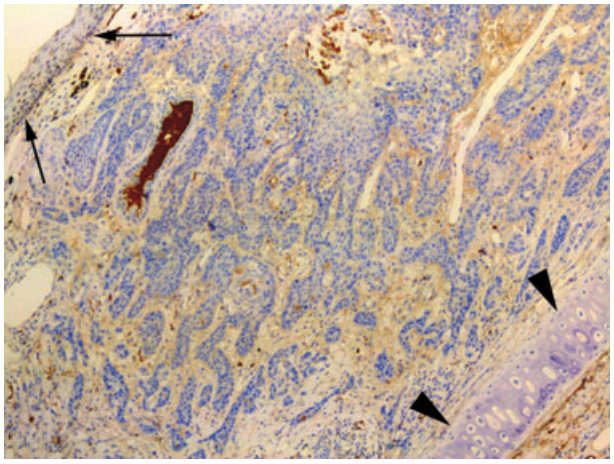

Figure 3. Photomicrograph of a feline squamous cell carcinoma removed from the pinna. A small proportion of the neoplastic cells display weak p16 immunoreactivity. Both non-neoplastic epidermis (arrows) and pinnal cartilage (arrowheads) are visible within the photomicrograph. Scattered immunoreactivity within the basal cell layer of the non-neoplastic epidermis is visible. Papillomaviral DNA was not amplified from this neoplasm. 3,3′-Diaminobenzidine substrate with haematoxylin counterstain. Magnification ×100.

($P < 0.001$; Table 2). There was no significant difference in the age of cats with SCCs containing PV DNA and cats with SCCs without PV DNA ($P = 0.98$).

Primers specific for FdPV-2 amplified DNA from 33 of 70 (47%) SCCs, while the consensus primers amplified PV DNA from seven (10%) SCCs. The consensus primers amplified DNA sequences that were >99% similar to FdPV-MY2[4] from five SCCs. The JMY2F/R primers amplified a section of the FdPV-MY2 sequence only from the same five SCCs as the consensus primers. The SCCs from which FdPV-MY2 was amplified included three from the nasal planum and SCCs from the eyelid and thigh. Both FdPV-2 and FdPV-MY2 were amplified from two nasal planum SCCs, while only FdPV-MY2 was detected in the other three. Increased p16 was present within all five SCCs that contained FdPV-MY2. Two SCCs contained a previously unreported sequence that was designated FdPV-MY3 (GenBank HM802139). The FdPV-MY3 sequence was most similar to FdPV-MY2, with 61% similarity over a 372 bp length. In comparison, FdPV-MY3 was only 58% similar to FdPV-2 over 372 bp. The specific JMY3F/R primers amplified the FdPV-MY3 sequence from the same two SCCs as the consensus primers as well as from one additional SCC. All three SCCs that contained FdPV-MY3 were from the nasal planum. One SCC contained both FdPV-MY3 and FdPV-2 DNA, while only FdPV-MY3 was present within the other two SCCs. Increased p16 was visible within all three SCCs that contained the FdPV-MY3 sequence. Papillomaviral DNA was not amplified from any negative control.

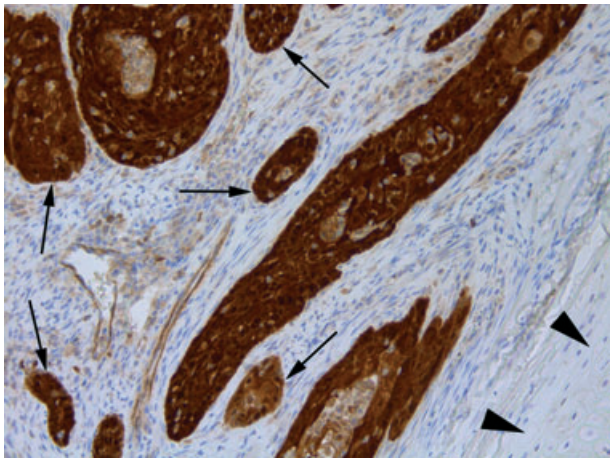

Figure 2. Photomicrograph of the same feline squamous cell carcinoma as in Figure 1. Consistent, intense p16 immunoreactivity is visible within the neoplastic cells (arrows). Pinnal cartilage is present within the photomicrograph (arrowheads). 3,3′-Diaminobenzidine substrate with haematoxylin counterstain. Magnification ×200.

Table 2. The presence of cellular $p16^{CDKN2A}$ protein (p16) and papillomaviral DNA within feline cutaneous squamous cell carcinomas

Location of squamous cell carcinoma	P16+ PV+	P16+ PV–	P16– PV+	P16– PV–	Total
All locations	32	7	6	25	70
UV-exposed skin	13	5	6	21	45
Nasal planum	7	2	2	5	16
Pinna	1	1	4	8	14
Eyelid	4	1	0	5	10
Third eyelid	1	1	0	3	5
UV-protected skin	19	2	0	4	25
Face	10	1	0	2	13
Digit	5	1	0	0	6
Thigh	2	0	0	2	4
Neck	2	0	0	0	2

Abbreviations: P16+, >90% of the neoplastic cells contain intense p16 immunoreactivity; P16–, <20% of the cells contain weak p16 immunoreactivity; PV+, papillomaviral DNA was amplified from the neoplasm; PV–, papillomaviral DNA was not amplified from the neoplasm; UV, ultraviolet light.

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Discussion

Neoplastic cells within 56% of the feline cutaneous SCCs contained increased p16. Increased p16 was reported in 38% of the neoplasms in a previous study of 18 feline cutaneous SCCs.[11] In the previous study, increased p16 was restricted to SCCs from UV-protected skin.[11] In the present study, although increased p16 was present more frequently in SCCs from UV-protected skin, increased p16 was also visible in 40% of UV-exposed SCCs. The p16 protein is important in controlling the cell cycle, and loss of normal regulation of p16 within a cell can promote neoplastic transformation. Dysregulation of p16 was frequently present within the feline SCCs. Therefore, loss of normal regulation of p16 may be an important mechanism of neoplastic transformation in feline cutaneous SCCs, especially in those in UV-protected skin. While mildly increased p16 is often present in human cutaneous SCCs, this is considered to be due to chronic UV exposure.[16,17] In contrast, as increased p16 was visible more frequently in feline SCCs from UV-protected skin, UV exposure is unlikely to be the cause of the markedly increased p16 visible within the SCCs in the present study.

Previous studies using consensus PCR primers amplified PV DNA from four of 22 (18%)[6] and nine of 39 (23%)[18] feline cutaneous SCCs, while PV DNA was amplified from 17 of 20 (85%)[3] and 12 of 18 (67%)[11] neoplasms using primers specifically designed to amplify FdPV-2. In the present study, specific PCR primers amplified PV DNA from 38 of 70 (54%) of the SCCs. Specific PCR primers appear to be a more sensitive method of amplifying PV DNA from formalin-fixed samples.[19] Therefore, it is probable that the variable rates of PV detection reported in the present and previous studies of feline cutaneous SCCs are due, at least in part, to the use of consensus and specific PCR primers.

Papillomaviral DNA can be amplified from the skin of 52% of clinically normal cats.[7] Therefore, although PV DNA was detected in 54% of the SCCs, it is difficult to differentiate between PV infection causing SCC development and the PV DNA being detectible within the SCC as an 'innocent bystander'. In the present study, PV DNA was detected more frequently in SCCs from UV-protected skin than in SCCs from UV-exposed skin. Protection from UV light does not promote cutaneous PV infection in either human[20] or feline[18] skin. This suggests that the different rates of PV detection within the two groups of SCCs are unlikely to be due to different rates of asymptomatic 'background' PV infection. Therefore, the higher rates of PV DNA detected in the UV-protected SCCs may have been because these SCCs are more frequently caused by PV infection, possibly as some UV-protected SCCs develop as a progression of a PV-induced viral plaque or BISC.

Oncogenic human PVs cause neoplasia by degrading Retinoblastoma protein (Rb), impairing normal cell cycle control and increasing p16.[9] This degradation of Rb is so consistent that the presence of increased p16 within a human oral SCC is considered synonymous with a PV aetiology.[21,22] In cats, intense p16 immunoreactivity and PV DNA were consistently present within a series of feline viral plaques and BISCs.[11] In the present study, increased p16 was associated with the presence of PV DNA within the feline cutaneous SCCs. This suggests that, as in human oral SCCs, PV infection of feline skin may cause SCC development by preventing normal Rb function. If the presence of increased p16 and PV DNA within a feline SCC does indicate that the neoplasm was caused by PV infection, the results of the present study suggest that approximately three-quarters of SCCs in UV-protected skin and one-quarter of SCCs in UV-exposed skin are caused by PV infection. The less frequent evidence of a PV aetiology in the SCCs from UV-exposed skin is consistent with these SCCs more often developing from actinic keratosis, caused by chronic UV exposure[1] rather than PV infection. Increased p16 indicates a PV aetiology within human oral SCCs.[21,22] However, investigations into the relationship between PV infection and p16 immunoreactivity in human skin lesions have produced contradictory results,[23,24] possibly due to the mild increase in p16 seen in UV-exposed skin.

Four SCCs from UV-protected skin did not contain increased p16. This suggests that loss of normal Rb function is common, but not essential, for the development of SCCs from UV-protected skin. Alternatively, due to the reliance on submission information to classify the SCCs in this study, it is possible that these four SCCs were removed from an area that had been misclassified as UV protected. Nine of the 39 SCCs with increased p16 did not contain detectible PV DNA. This could be evidence that PV infection is not the cause of the increased p16 observed within the SCCs. However, it is also possible that the nine SCCs contained PV DNA that could not be amplified due to damage caused by formalin fixation. Alternatively, previous studies of feline SCCs have detected multiple different PV types, including human PVs.[6,18] Therefore, it is possible that the nine SCCs contained PV DNA, but the DNA was not amplified by the PCR primers used in this study because it was from other PV types. Six of the 38 SCCs that contained amplifiable PV DNA did not contain increased p16. This suggests that PVs can be present within a feline SCC without increasing p16. It is possible that disruption of normal Rb function by the PV is necessary to cause the development of a clinically visible lesion. This hypothesis is supported by the consistently increased p16 previously observed in feline viral plaques and BISCs.[11]

Felis domesticus papillomavirus-2 was the most frequently detected PV in the 70 feline cutaneous SCCs in the present study. *Felis domesticus* papillomavirus-2 is thought to be the cause of both viral plaques and BISCs.[3,4] Therefore, the detection of FdPV-2 within 72% of the SCCs from UV-protected skin further supports the hypothesis that these SCCs may be caused by PV infection and may develop as a progression of a PV-induced skin lesion. A previous study detected FdPV-2 DNA more frequently in feline cutaneous SCCs than in non-SCC skin lesions.[3] *Felis domesticus* papillomavirus-2 was initially detected in a series of feline BISCs,[13] has been fully sequenced,[25] and is classified as a dyotheta-PV.[26]

The FdPV-MY2 sequence was amplified from five SCCs in the present study. This sequence was initially detected in one of a series of 14 feline viral plaques.[4]

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Three of the SCCs contained a previously unreported DNA sequence that was designated FdPV-MY3. Definitive classification of this PV was not possible because the complete L1 gene was not determined.[26] However, as the sequence was only 61% similar to other PV sequences, FdPV-MY3 may represent a novel PV type.[26] The inconsistent detection of FdPV-MY2 and FdPV-MY3 within the feline SCCs suggests that they could infect feline skin without causing clinically visible disease. However, five SCCs contained increased p16 and either FdPV-MY2 or FdPV-MY3, but not FdPV-2 DNA. This suggests that both FdPV-MY2 and FdPV-MY3 can increase p16. It is interesting that three of five FdPV-MY2, and all three FdPV-MY3, sequences were detected within nasal planum SCCs. Some PV types have been reported preferentially to infect specific sites within the body.[27] If these PVs do preferentially infect the nasal planum and inhibit normal Rb function, they could be the cause of the higher rate of increased p16 within SCCs from the nasal planum than within SCCs from the pinna.

In conclusion, the feline cutaneous SCCs were subdivided into those from UV-protected and those from UV-exposed skin. Squamous cell carcinomas from UV-protected skin more frequently contained PV DNA than SCCs from UV-exposed skin. The UV-protected SCCs also more frequently contained increased p16. Increased p16 may indicate loss of normal Rb function,[28] suggesting that this could be common within SCCs from UV-protected skin. In human oral SCCs, increased p16 is indicative of a PV aetiology.[21] Likewise, increased p16 was more frequently present in feline SCCs that contained PV DNA. The frequent detection of increased p16 and PV DNA within SCCs from UV-protected skin suggests that these SCCs may be caused by PV infection and may develop as a progression from a PV-induced viral plaque or BISC. This is further supported by the frequent detection of the same PV that is thought to cause viral plaques and BISCs[3,4] within SCCs from UV-protected skin. Increased p16 and PV DNA was less frequently detected in SCCs from UV-exposed skin, presumably because these normally develop from actinic keratosis rather than a PV-induced lesion. Although associations between PVs and feline SCCs have been previously reported,[3] this study is the first to demonstrate differences in the presence of PV DNA and p16 within SCCs from UV-protected skin and within SCCs from UV-exposed skin. The results suggest that PV infection could influence the development of a proportion of feline SCCs, especially those within UV-protected skin. Loss of normal Rb function appears to be common in feline cutaneous SCCs and may be caused by PV infection.

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Résumé Les carcinomes épidermoïdes (SCCs) sont des tumeurs cutanées félines fréquentes. Alors que l'exposition à la lumière UV (ultraviolet) provoque certains SCCs, d'autres se développent sur la peau non-exposée UV. Chez les chats, les papillomavirus (PVs) causent des plaques virales et des carcinomes de Bowen *in situ* (BISCs). Comme les deux peuvent évoluer en SCC, il a été suggéré que les SCCs des zones cutanées non-exposées aux UV pourraient résulter de la transformation néoplasique d'une lésion liée au PV. Pour explorer cette hypothèse, une PCR a été utilisée pour amplifier l'ADN de PV à partir de 25 SCCs de peau non-exposée aux UV et de 45 SCCs de peau exposée aux UV. Les PVs oncogéniques humains provoquent des tumeurs par des mécanismes qui augmentent la protéine $p16^{CDKN2A}$(p16). Comme une augmentation de p16 est présente dans les plaques virales félines et les BISCs, l'immunohistochimie a été utilisée pour détecter p16 au niveau des SCCs. L'ADN papillomaviral a été amplifié à partir de 76% des SCCs non-exposés aux UV mais seulement de 42% des SCCs exposés aux UV. Une p16 augmentée était présente dans 84% des SCCs non-exposés, mais juste 40% des SCCs exposés aux UV. La détection plus fréquente d'ADN de PV et une augmentation de p16 au niveau des SCCs non-exposés aux UV supportent le développement de lésion induite par PV. Une augmentation de p16 et d'ADN de PV était moins fréquente avec les SCCs exposés aux UV, probablement parce qu'ils se développent à partir de kératose actinique plutôt que de lésion liée à PV. Les résultats supportent l'hypothèse que certains SCCs cutanés félins sont dus à une infection à PV et suggèrent que les PVs pourraient causer une néoplasie par des mécanismes élevant aussi p16.

Resumen El carcinoma de células escamosas (SCCs) es una neoplasia común en la piel de gatos. Si bien la exposición a radiación ultravioleta (UV) causa algunos casos de SCCs, existen algunos que se desarrollan en piel protegida de la radiación. En gatos, los virus papiloma (PVs) causan placas virales y carcinomas *in situ* de tipo Bowen (BISCs). Ya que ambos pueden progresar a SCC, se mantiene la hipótesis de que los SCCs en zonas protegidas de radiación UV puede representar una transformación neoplásica de una lesión inducida por PV. Para investigar esta hipótesis, se utilizo PCR para amplificar DNA de PV de 25 SCCs de zonas no protegidas y 45 de zonas expuestas a radiación UV. Los PVs humanos oncogénicos causan neoplasia mediante mecanismos que también aumentan la expresión de proteína $p16^{CDKN2A}$ (p16). Ya que se observa incremento de p16 en las placas virales felinas y en BISCs, se utilizó inmunohistoquímica para detectar p16 en los SCCs. Se amplificó DNA de virus papiloma de un 76% de SCCs protegidos de radiación UV, pero sólo de un 42% de SCCs expuestos a radiación UV. Se observó un incremento de p16 en un 84% de los SCCs protegidos de radiación UV pero sólo en un 40% de los SCCs expuestos a radiación UV. La detección con mayor frecuencia de DNA de PV y la expresión aumentada de p16 en los SCCs protegidos de radiación UV apoya la hipótesis de que algunos se desarrollan a partir de una placa vírica o de BISC. El PV-2 de *Felis domesticus* se asocia como la causa de las placas víricas y de BISCs. Este papilomavirus se detecta con mayor frecuencia en los SCCs protegidos de la radiación UV, apoyando el desarrollo a partir de una lesión inducida por PV. El incremento de la expresión de p16 y de la presencia de DNA de PV fue menos frecuente en SCCs expuestos a radiación UV, presumiblemente porque estos se desarrollan a partir de queratosis actínica en lugar de una lesión producida por PV. Los resultados apoyan la hipótesis de que algunos SCCs felinos son causados por infección por PVs y sugieren que los PVs puede causar neoplasia mediante mecanismos que también incrementan la expresión de p16.

Zusammenfassung Plattenepithelkarzinome (SCCs) sind häufige Hauttumoren bei der Katze. Während die Exponierung zu ultraviolettem (UV) Licht manche SCCs verursacht, entwickelt sich auch eine Untergruppe in UV-geschützter Haut. Bei Katzen verursachen Papillomaviren (PVn) virale Plaques und Bowen Carzinoma *in situ* (BISCs). Da sich beide zu einem Plattenepithelkarzinom weiterentwickeln können, wurde die Hypothese aufgestellt, dass SCCs in UV-geschützter Haut die neoplastische Transformation einer PV-induzierten Veränderung repräsentieren können. Um diese Hypothese zu untersuchen, wurde eine PCR verwendet, um PV-DNA aus 25 UV-geschützten und 45 UV-exponierten SCCs zu amplifizieren. Onkogene humane PVn verursachen die Bildung von Neoplasien durch Mechanismen, die auch das p16CDKN2A Protein (p16) erhöhen. Da erhöhtes p16 in felinen viralen Plaques und in BISCs vorkommt,

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wurde mittels Immunhistochemie versucht, p16 innerhalb der SCCs zu finden. Die DNA von Papillomavirus wurde aus 76% der UV-geschützten, aber nur aus 42% der UV-exponierten SCCs amplifiziert. Erhöhte p16 kam in 84% der UV-geschützten, aber nur in 40% der UV-exponierten SCCs vor. Der häufigere Nachweis von PV-DNA und erhöhtes p16 in den UV-geschützten SCCs unterstützt die Hypothese, dass einige sich aus PV-induzierten Plaques oder BISC entwickeln. Man glaubt, dass PV-2 von *Felis domesticus* virale Plaques und BISCs verursacht. Dieses PV wurde am häufigsten innerhalb der UV-geschützten SCCs nachgewiesen, was die Hypothese der Entwicklung aus einer PV-induzierten Läsion unterstützt. Erhöhtes p16 und PV-DNA waren weniger häufig in UV-exponierten SCCs nachzuweisen, vermutlich da sich diese eher aus einer aktinischen Keratose, statt aus einer PV-induzierten Läsion entwickelten. Diese Ergebnisse unterstützen die Hypothese, dass einige feline kutane Plattenepithelkarzinome durch eine PV Infektion verursacht sind und geben Hinweise darauf, dass PVn Neoplasien durch Mechanismen, die p16 erhöhen, verursachen können.

要約　扁平上皮癌(SCCs)は一般的な猫の皮膚腫瘍である。一部の SCC は紫外線(UV)への暴露によりが生じるにもかかわらず、一部は UV から保護された皮膚にも発生する。猫ではパピローマウイルス　(PVs) がウイルス性局面やボーエン様上皮内癌 (BISCs) の原因となる。それぞれが SCC に進行する可能性があるが、UV から保護された皮膚の SCC は PV 誘発性病変の腫瘍性形質変換である可能性をしめすという仮説がある。この仮説の調査のため、を 25 頭の UV 被保護 SCC と 45 頭の UV 暴露 SCC から PV 遺伝子を増幅する PCR を行った。腫瘍原性のヒト PV は、$p16^{CDKN2A}$ 蛋白(p16)を増幅することによって腫瘍が発生する。猫ウイルス性局面や BISC では p16 が増加しているため、免疫組織化学を用いて SCC 材料の p16 を検索した。パピローマウイルス遺伝子は UV 被保護の SCC の 76%から増幅されたが、UV 暴露 SCC では 42%のみから増幅された。P16 の増加は 84%の UV 被保護の SCC で認められたが、UV 暴露 SCC では 40%しか認められなかった。PV の検出と p16 の増加が UV 被保護 SCC でより頻繁にみられたことは、一部の SCC が PV 誘発性局面や BISC から発生したという仮説を支持するものである。*Felis domesticus* PV-2 はウイルス性局面や BISC を起こすと考えられている。この PV は PV 誘発性病変から発症した UV 被保護 SCC で最も頻繁に検出された。P16 の増加と PV 遺伝子は UV 暴露 SCC ではあまり見られないため、 PV 誘発性病変からではなく光線角化症から発生するのではないかと推定される。この結果は一部の猫の皮膚 SCC が PV 感染によって起きるという仮説を支持し、PV が p16 の増加を生じる機序によって腫瘍を発生させる可能性を示唆している。

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