Overview of urogenital carcinoma studies in California sea lions

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Categories : General, Vets

Date : October 31, 2016

ABSTRACT

Urogenital carcinoma is an important disease of wild California sea lions, diagnosed in 26% of adult animals necropsied at a marine mammal rescue centre in California. Similar to other cancers, the likely multifactorial aetiology makes it challenging to identify risk factors. Investigations into the disease have reported viral infection, environmental contaminants and genetic factors as potential risk factors.

This article presents a review of work into a genetic basis of the disease and discusses previously reported findings regarding other potential aetiologies.

Wild California sea lions (CSLs) are found along the west coast of North America, from the Baja California Peninsula in Mexico to British Columbia. Their population is large, estimated at more than 200,000 animals[1].

CSLs are a charismatic, social animal, grouping together at haul out sites, such as Pier 39 in San Francisco, which makes them a popular tourist attraction in the area.

Figure 1a. Clinical signs in a female California sea lion suffering from urogenital carcinoma.

Perineal and hind flipper oedema. Photo taken during a necropsy examination at The Marine Mammal Center.

Their lifespan in the wild has been reported as being between 15 to 24 years, with sexual maturity of both sexes reached at 4 to 5 years. However, they may not breed until they are older[1].

Gestation length is 11 months, which includes a three-month period of embryonic diapause, with pups born from May to July[2,3].

Carcinoma in CSLs

The Marine Mammal Center (TMMC) in California specialises in the treatment and rehabilitation of marine mammals. In addition, it is a research institute, monitoring the health of animals and identifying trends.

Full necropsies are performed on all animals that die or are euthanised, and samples are collected and archived for future and ongoing projects, collaborating with many other research institutes.

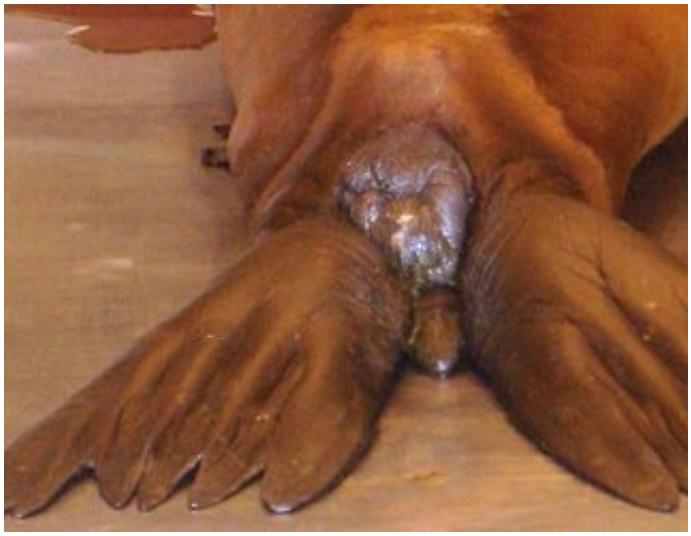

Figure 1b. Clinical signs in a female California sea lion suffering from urogenital carcinoma, in this case visible emaciation. Photos taken during a necropsy examination at The Marine Mammal Center.

In 1996, Frances Gulland, TMMC's senior scientist, reported 18% of adult CSLs necropsied between 1979 and 1994 were suffering from metastatic carcinoma of presumed urinary tract origin[4]. Both males and females were affected, and were, on average, approximately eight years old. Since then monitoring has continued, with an increase in prevalence to 26% seen between 1998 and 2012.

It should be noted, however, this is 26% of the adult animals necropsied at TMMC and not 26% of the population. Regardless, the findings suggest it is an important cause of morbidity and mortality in the wild population.

Clinical signs and pathology

Affected CSLs present with a variety of clinical signs, including cachexia, hind flipper paresis, ascites, hind flipper and perineal oedema and, in severe cases, rectal prolapse[4] (**Figure 1**)[5].

On gross pathology, abnormalities are varied and include lesions in the genital tract, with metastasis to abdominal and pelvic lymph nodes, and distant sites such as the liver, lungs and spleen (**Figure 2**). The renal system is also frequently affected with metastasis to the kidneys.

Bladder distension and, in some cases, hydroureter and hydronephrosis are also reported[4]. The presence of transitional cells on histopathological analysis in earlier work suggested a urinary tract origin, but further histopathological analysis of the genital tract of affected animals has revealed the presence of intraepithelial neoplasia (IEN)[4,6]. IEN is associated with cervical cancer in humans, with three different grades of cervical intraepithelial neoplasia (CIN) reported according to the level of cellular dysplasia identified[7].

Figure 2a. Gross pathological lesions seen on necropsy examination of a California sea lion with metastatic urogenital carcinoma, including here numerus metastatic lesions in the liver. Photo taken during necropsy examination at The Marine Mammal Center.

In CSLs the grading system uses two levels – low-grade intraepithelial neoplasia and high-grade intraepithelial neoplasia. The difference is due to lesions not being restricted to the cervix, but also being identified in the vagina, penis and prepuce[8].

Lesions have also been seen in urethral tissue; therefore, at present, the point of origin of the disease still requires clarification[8,9] and thus the term urogenital carcinoma (UGC) is used to describe it. The majority of UGCs are squamous cell carcinomas, although adenocarcinomas are also reported[4,8].

Aetiology

The multifactorial aetiology of neoplasia complicates the identification of predisposing factors. To investigate potential risk factors for the development of UGC in the CSL, the Sea Lion Cancer Consortium was established in 2010 to encourage collaboration and pooling of ideas between institutes.

To date, four main areas have been investigated – hormone receptor expression, genetics, herpesvirus infection and contaminant levels. A collaboration between the TMMC; the Sea Mammal Research Unit (SMRU) at the University of St Andrews; The Pirbright Institute; the Unit for Basic and Applied Microbiology, Querétaro, Mexico; and The Moredun Institute allowed researchers to concentrate predominantly on a genetic basis of the disease.

Genetics

Investigations into a genetic basis of UGC have looked into two main areas – major histocompatibility complex class II (MHC II) allele diversity, which identified the presence of certain MHC II alleles predisposed animals to cancer[10] and the effect inbreeding has on the presence of UGC, which formed the basis of the study.

Previous work using a measure of inbreeding called internal relatedness and involving the genotyping of 371 animals at 11 polymorphic microsatellite loci, 13 of which had carcinoma, identified more inbred animals had an increased susceptibility to carcinoma[11,12]. However, further work investigating this suggested the relationship was driven by one particular microsatellite, PV11.

To investigate this phenomenon further, data obtained from preliminary studies undertaken by Karina Acevedo-Whitehouse was expanded during the study by the addition of a further 113 samples – in total, 383 samples consisting of 274 controls (animals dying or euthanised due to a condition other than UGC) and 109 cases.

All the samples were collected during necropsy examinations at TMMC. Extraction of genomic DNA from skin was carried out, followed by multiplex PCR amplifying three microsatellite markers (one of which was PV11) and analysed via automated capillary electrophoresis. Statistical analysis

of the results from the samples as to the effect of genotype at the three microsatellite loci on the presence of UGC revealed homozygosity of the PV11 microsatellite was significantly associated with the presence of UGC (odds ratio; 1.62, p=0.04)[13].

A combination of comparative genomics using the dog genome and molecular methods was used to establish the position of PV11 in the CSL genome. Although the CSL genome has been sequenced, it has not been assembled; therefore, an analysis of the dog genome (which shares a common ancestor with pinnipeds[14]) was carried out[13]. This was followed by a southern blot using two molecular probes to support the findings of the comparative genomic study.

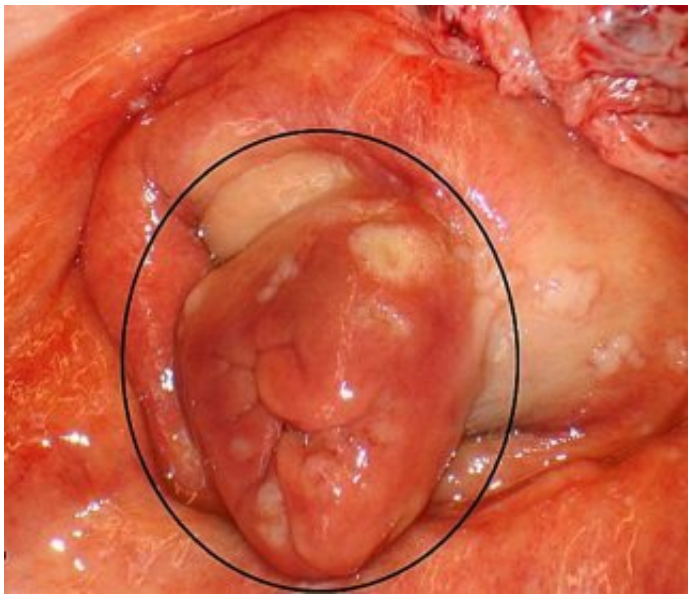

Figure 2b. Gross pathological lesions seen on necropsy examination of a California sea lion with metastatic urogenital carcinoma. Ulcerative lesions of the cervix indicative of urogenital carcinoma (cervix circled). Photo taken during necropsy examination at The Marine Mammal Center.

Comparative genomics led the researchers to believe the PV11 microsatellite was located within intron 9 of a large gene called heparanase 2 (HPSE2)13. The subsequent southern blot using CSL genomic DNA along with two molecular probes; one targeting exon 9 of HPSE2 and the other the region flanking the PV11 microsatellite, verified this[12].

The researchers confirmed transcription of the gene in genital tract tissue from animals of different PV11 genotype and disease state, and revealed the presence of numerous splice variants[13].

To confirm expression of the gene at the protein level in genital tract tissue, samples taken from animals of different PV11 genotype and disease state were examined by undertaking an immunohistochemical (IHC) study using a polyclonal goat IgG antibody raised against a peptide of human heparanase 2 (HPA2; C-17, Santa Cruz Biotechnology). This antibody is reported in the data sheets as being suitable for detecting HPA2 in species other than humans, including dogs;

therefore, it was deemed suitable for the study.

Interestingly, labelling of HPSE2 protein (HPA2) did occur, but only in animals suffering from UGC and of only one PV11 genotype[13]. This finding confirmed the PV11 microsatellite and HPSE2 gene were linked and brought the conclusion this is the first cancer gene to be identified in a wildlife species[13].

Interestingly, the protein HPA2 has been noted to share around 35% amino acid sequence identity over the coding regions with the protein heparanase 1 (HPA1) encoded by the well-known oncogene heparanase (HPSE)[15]. Furthermore, studies in humans with cervical cancer have identified increased labelling of HPA2 in cervical tissue with increasing severity of disease[16].

Other areas of investigation

Hormone receptor expression

Increased expression of the hormone receptors progesterone receptor (PR) and oestrogen receptor alpha (OR?) in canine mammary tissue have been noted to be associated with a better prognosis in animals with mammary tumours[17].

IHC studies into expression of PR and OR? in urogenital tract tissue from CSLs with varying levels of cellular dysplasia and neoplastic lesions identified a reduced expression of OR? in more affected tissue[8].

Herpesvirus

A novel herpesvirus, distinct from other pinniped herpesviruses, has been found in the genital tract of CSLs with UGC and is considered a potential aetiological agent in the development of UGC[5,18].

The virus, named otarine herpesvirus-1 (OtHV-1), is identified as a gammaherpesvirus with phylogenetic analysis using the DNA polymerase gene placing it close to human herpesvirus 8 (HHV-8) on the phylogenetic tree[6,18].

HHV-8, also known as Kaposi's sarcoma-associated herpesvirus, is an oncogenic virus and the aetiological agent in the neoplastic condition Kaposi's sarcoma, seen more commonly in human immunodeficiency virus-positive individuals[19]. The potential involvement of OtHV-1 in the aetiology of UGC in the CSL is an area of continued investigation.

As yet, an association with infection with a papillomavirus, which is an important risk factor for cervical cancer in humans where infection with human papillomavirus 16 and 18 in particular are highlighted[20], has not been identified.

Exposure to contaminants

Environmental contaminants in the form of organochlorines (OC) and polycyclic aromatic hydrocarbons (PAH) are believed to contribute to the high number of tumours found in Beluga whales (Delphinapterus leucas) from the highly polluted St Lawrence River estuary in Canada[21,22].

OC and PAH pollutants have been identified in coastal waters around California[23,24] and an increased level of OCs were found in the blubber of CSLs with UGC compared to non-cancer animals, suggesting an association with UGC in the CSLs and environmental OC contaminants[25]. Additional work is ongoing in this area.

Conclusion

UGC in CSLs is a complex condition with a number of potential aetiologies contributing to its development.

Age of occurrence in CSLs (middle-aged animals) mirrors cervical cancer in humans that predominantly occurs in adults, but not necessarily aged women[26,27]. This, along with the presence of IEN lesions, the association with a virus and labelling of the HPA2 protein in cervix tissue, offer interesting parallels.

These findings made the researchers consider the CSL as a potential useful wild animal model of carcinogenesis, allowing the study of risk factors (environmental, genetic and infectious) in the development of neoplasia, which could have important implications on humans and terrestrial mammals28.

Acknowledgements

Ailsa Hall (University of St Andrew SMRU Scottish Oceans Institute), John Hammond (The Pirbright Institute), Frances Gulland, (TMMC), Karina Acevedo-Whitehouse (Unit for Basic and Applied Microbiology, Querétaro, Mexico) and Mark Dagleish (The Moredun Research Institute Pentlands Science Park, Midlothian).

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