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PATHOLOGICAL AND MOLECULAR INSIGHTS OF ANIMAL MELANOMA CELL CULTURES - A COMPREHENSIVE REVIEW

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Abstract

Melanoma is a particular neoplasm arising from the unregulated proliferation of melanocytes. It affects both humans and different animal species that develop this tumour spontaneously, including canines, equines, and rarely felines. Understanding the pathology and molecular biology of melanoma can be enhanced by studying both two-dimensional and three-dimensional melanoma cell cultures from various species, some animals (e.g. dogs) sharing multiple physiopathological mechanisms with human melanoma. Moreover, established mouse melanoma cell lines from genetically modified models are commonly used for molecular characterization and utility in therapeutic testing, while noting limitations posed by genetic differences. Recent studies regarding isolation protocols for cultivating neoplastic melanocyte cultures show a significant variation including fine-needle aspiration, tissue excision, and enzymatic digestion. Hence, comparative genetic analyses indicate similarities between animal and human melanoma cells, especially regarding mutations in the BRAF and NRAS oncogenes. This review highlights the relevance of melanoma cell cultures across species as significant in vitro models for advanced cross-species melanoma research, enhancing insights into neoplastic initiation and progression and ultimately contributing to improved diagnostic and therapeutic approaches in veterinary and human pathology and oncology.

Key words: *melanoma, melanocytes, cell cultures, oncogenes, animal models.*

INTRODUCTION

Melanoma is a particularly aggressive type of cancer that arises from the uncontrolled growth and neoplastic transformation of melanocytes, which are cells that originate in the basal layer of the epidermis and derive from multipotent neural crest cells (melanoblasts) (Horak et al., 2019; Sforna et al., 2021). Although melanocytes are also naturally present in various parts of the body, such as the iris and inner ear, cutaneous malignant melanoma (CMM) remains the most commonly encountered form in human oncology (Aktary et al., 2023). Among various types of melanomas, mucosal ones are relatively rare in humans, but still considered a highly metastatic and aggressive variant of this neoplasm (Segaoula et al., 2018). Moreover, despite its rarity, uveal melanoma represents one of the most prevalent intraocular tumours in both human and veterinary oncology (Uner et al., 2022). Thus, the development of predictive animal models and cell cultures that accurately mimic the pathogenesis and molecular alterations associated with various types of melanomas presents substantial challenges for further genetic and even clinic investigations.

Melanoma also affects various animal species, including dogs (~7% of malignant pathologies, ~30% of oral tumours), horses, and, to a lesser extent, cats (less than 1%), frequently arising spontaneously (Polton et al., 2024; He at al., 2024; Cristian et al., 2023; Hrițcu et al., 2023; Aktary et al., 2019). This phenomenon of interspecies occurrence presents a distinctive opportunity for the comparative study of melanoma, facilitating the development of improved treatment options for both human and animals.

Melanomagenesis, the transformation of melanocytes into melanoma cells, which usually begins with the benign proliferation of melanocytes, leading to the formation of a nevus, where melanocytes cluster and lose contact with surrounding keratinocytes (Aktary et al., 2019; Horak et al, 2019). These nevus cells may subsequently stop proliferation and enter senescence; or bypass senescence and start actively proliferation and basement membrane invasion (Gao et al., 2024). This process involves alterations in key cellular functions, including proliferation, immortalization, epithelial-mesenchymal transition, and migration, as well as changes in molecular signalling pathways, cell cycle regulation, and adhesion (Aktary et al., 2019). Activation of tyrosine kinase receptors such as KIT, MET, and RET by their respective ligands (SCF - Stem Cell Factor, HGF - Hepatocyte Growth Factor, and GDNF - Glial cell line-derived neutrophic factor) can trigger various signalling pathways (e.g., ERK1/2 MAP-kinase - Extracellular signal-regulated kinase 1/2, mitogen-activated protein kinase; PI3/PTEN-AKT - Phosphoinositide 3-kinase/Phosphatase and Tensin homolog/Protein kinase B; WNT/β-catenin) that play critical roles in melanoma development, both *in vivo* and *in vitro* (Aktary et al., 2019; Sforna et al., 2021).

In recent years, considerable progress has been made in the establishment and characterization of melanoma cell lines, which have become essential for functional assays, molecular characterization, and therapeutic testing. Among the various models used in melanoma research, mouse melanoma cell lines have gained particular prominence due to their genetic similarity to human melanomas and the ease with which they can be genetically modified. However, it is crucial to acknowledge the limitations that arise from the genetic differences between species (Aktary et al., 2023; Uner at al., 2022; Kuzu et al., 2015).

Thus, the role of animal models in melanoma research is no longer limited to murine species; it consists of a bigger spectrum of animals which naturally develop melanomas. Notably, canine melanoma, especially the mucosal oral and acral variants, exhibits multiple shared molecular and pathological mechanisms with human melanoma, including genetic alterations in key oncogenes such as BRAF, NRAS, PTEN, and KIT and involving the same signalling pathways as MAPK, PI3K and WNT (Gao et al., 2024; Uner et al., 2022; Nishiya et al., 2016).

Melanoma cell cultures allow researchers to thoroughly analyse the pathways involved in tumoural progression by providing controlled environments for studying both the molecular and cellular levels of melanoma (He at al., 2024; Marconi et al., 2018). The development of both two-dimensional (2D) and three-dimensional (3D) cell cultures has been noted, the 3D models allowing more relevant representations by mimicking the tumour *in vivo* architecture. Furthermore, different methods have been utilized in recent studies for isolating neoplastic melanocytes, including tissue excision, fine-needle aspiration, and enzymatic digestion (Lo-Giudice et al., 2024; Correa et al., 2009). Therefore, the establishment of permanent animal melanoma cell lines has represented an important advancement in melanoma research, enabling in-depth functional further assays.

This review offers a thorough examination of the pathological and molecular insights derived from mammalian melanoma cell cultures, underscoring their translational significance for both veterinary and human melanoma research.

MATERIALS AND METHODS

An extensive bibliographic study was conducted to select scientific articles from the Web of Science, Scopus, Science Direct and PubMed databases, focusing on key words such as "animal melanoma cell cultures", "canine melanoma cell cultures", "murine melanoma cell cultures", "equine melanoma cell cultures", "feline melanoma cell cultures", "swine melanoma cell cultures", "melanoma molecular biology", "melanoma pathology" and "comparative oncology". A noticeable increase in publications related to these topics, particularly in studies conducted on murine, canine and equine models, has been observed since the early 2000s to the present year (2025), highlighting a growing interest among researchers from various fields. This increasing trend emphasizes the importance of mammalian permanent melanoma cell cultures in advancing the understanding of melanoma biology, tumour microenvironment, and further developing of new therapeutic strategies.

RESULTS AND DISCUSSIONS

In the last decades, significant efforts have been invested in the development of suitable animal models for the study of various types of melanomas. Thus, murine melanoma cell lines, such as B16-F10 and B16-LS9, derived from genetically engineered models, are widely utilized nowadays in melanoma research (for both cutaneous and uveal melanomas), due to their genetic similarity to human melanoma, enabling investigations into tumour progression, metastasis, and the tumour microenvironment (Olbryt et al., 2006; Aktary et al., 2019). Their usage has previously advanced the investigation of the dysregulated PI3K/AKT and MAPK signalling pathways, which play particular roles in melanoma development, being also encountered in the pathogenesis of human melanoma (Richards et al., 2020).

In contrast with human sporadic melanoma, in murine melanoma lines, genetic studies have revealed that no BRAF (v-Raf murine sarcoma viral oncogene homolog B) mutation was involved (Melnikova et al., 2004). However, mutations of the MAPK pathway (BRAF V600E, HRAS - Harvey Rat Sarcoma Viral Oncogene Homolog, NRAS - Neuroblastoma RAS Viral Oncogene Homolog) can be generated in murine melanocytes through genetic engineering (Aktary et al., 2023).

Regarding canine melanoma, dogs serve as an important model for studying melanoma due to the natural spontaneous incidence of this neoplasm in dogs. Mucosal melanomas are recognized for being particularly aggressive and for their tendency for early metastasis (Gao et al., 2020; Pérez-Santana et al., 2024). Additionally, ocular melanoma is the most common primary intraocular tumour encountered in this species, sharing multiple molecular characteristics with human uveal melanoma, although in dogs these melanomas usually arise in the anterior uvea site, while in humans they commonly develop in the choroid (Polton et al., 2024; Singh et al., 2014; Nishiya et al., 2016).

Canine and human melanoma cell lines share mutations in key carcinogenic pathways, including the MAPK (mitogen-activated protein kinase; 43% of canine mucosal melanoma) and PI3K/AKT/mTOR (phosphoinositide 3-kinase/protein kinase B) signalling cascades, which play crucial roles in tumour proliferation and progression (He et al., 2024; Aktary et al., 2019). In addition, both species have comparable sensitivity in cell cultures to therapeutic inhibitors (e.g. rapamycin and AZD6244) targeting these previously mentioned carcinogenic pathways (van der Weyden et al., 2016). Mutational activation of the proto-oncogene BRAF, NRAS, KRAS (Kirsten Rat Sarcoma Viral Oncogene Homolog), PTEN, and KIT has been identified in both species, although with varying frequencies, being more common in sun-damaged skin melanomas in humans (Dobrin & Militaru, 2023; van der Weyden et al., 2020). In contrast, oncogene BRAF V600E mutation is frequently encountered in human cutaneous melanoma due to the mutagenesis produced by ultraviolet irradiation, but it is less common in canine melanoma (~6%). However, canine melanoma often exhibits NRAS and KIT mutations, which are also found in certain subtypes of non-UV induced human melanoma, particularly mucosal and acral melanomas (Gao et al., 2024).

The establishment of two canine oral melanoma cell cultures, Ocr_OCMM1X and Ocr_OCMM2X, has been obtained from surgically excised melanocytic lesions localized within the oral cavity (Segaoula et al., 2018). Furthermore, a mucosal melanoma cell line, COMM6605, has been developed from a fresh tumoral tissue sample obtained from a male Bichon Frise dog. This process has employed enzymatic digestion techniques, specifically utilizing type IV collagenase within the cellular medium to facilitate tissue breakdown (Li et al., 2024).

In an additional study involving five canine melanoma cell cultures, two of which were derived from cutaneous melanomas and other two were mucosal melanomas, has showed that enzymatic digestion is preferred for obtaining a larger number of cells (Sforna et al., 2021).

Regarding collection techniques, fine-needle aspiration (FNA) was also utilized in some oral melanoma cases, where samples has been obtained from primary tumours and/or lymph nodes metastasis (Lo Giudice et al., 2024). While FNA technique provides a minimally invasive method for cell collection, it still presents limitations regarding collecting an adequate cell number. However, direct fine-needle aspiration from the inner part of the tumour mitigated has helped in obtaining cleaner samples, lowering the bacterial contamination. Additionally, three-dimensional (3D) cell cultures obtained with FNA have been established to better replicate the tumour microenvironment and enhance the study of cellular interactions *in vitro* in the future. Spheroids, which are fundamental 3D clusters created by aggregating cells of a single type, have been successfully developed from canine oral melanoma metastases, forming aggregates of round to polygonal neoplastic cells, while organoids, which consists of multiple types of cells and present self-differentiation capabilities that enable them to develop structures resembling tissues and organs, have been established from both primary melanomas and metastases (Lo Giudice et al., 2024; Lee et al., 2023).

In feline population, melanoma is an uncommonly encountered tumour, representing less than 1%, the most frequently reported variant being the ocular melanoma which commonly affects the uveal tract and the iris (Polton et al., 2024). Unfortunately, the only notable characterisation studies of feline melanoma cell cultures have been conducted in 1983 by Rasheed S. and later, in 2002, by Mayr B. and his collaborators.

As for translational relevance, feline *in vivo* models for uveal melanoma have been developed by inoculating a strain of FeSV (Feline Sarcoma Virus) in the iris, obtaining tumours in 90% of the inoculated cats, illustrating atypia of melanocytes in the uveal site (Uner et al., 2022).

Previous molecular studies of feline uveal melanoma showed that the most commonly oncogenes mutations known in human ocular melanomas (BRAF V600E, GNAQ - Guanine Nucleotide-Binding Protein Alpha-Q, NRAS, KIT, MEK1 - Mitogen-activated protein kinase kinase 1) are rarely encountered in feline melanoma samples, only one case of feline cutaneous melanoma with mutation of BRAF oncogene being described (Kuroki et al., 2024; Kayes & Blacklock, 2022).

Equine melanomas can spontaneously occur, being distinguished 5 types of melanocytic tumours in this species: melanocytoma, dermal melanoma, dermal melanomatosis, anaplastic malignant melanoma and benign cutaneous/mucosal melanoma (Dobrin et al., 2024; Aktary et al., 2019; Chapman et al., 2009). While human melanoma is commonly associated with UV irradiation and unfrequently genetic mutations, equine melanomas, most encountered in aging grey-coated horses, are commonly strongly linked to a genetic alteration in the STX17 gene (which encodes for *syntaxin 17*) (Seltenhammer et al., 2014). It has been previously noted that this mutation implies further activation of the ERK1/2 MAPK pathway, pointing out the importance of this signalling pathway in melanoma development regardless the species (Pimenta et al., 2023; van der Weyden et al., 2020).

One study revealed that in three equine melanoma cell lines (MelDuWi, eRGO1, eRGO6) mutant BRAF oncogene was detected, along with one encountered mutation of KRAS proto-oncogene in MelDuWi cell line (Gao et al., 2024). Additionally, such as in human and canine melanoma, in 2019, Wong. K. et al. demonstrated through next generation sequencing on equine melanomas the presence of mutations in the same oncogenes (NRAS, TP53 - tumour protein p53, KIT, BRAF) that could activate the ERK pathways implicated in melanomagenesis. Hence, the same signalling cascade and mutations of proto-oncogenes are involved in both human, canine and equine melanomas.

Regarding the protocols for obtaining equine melanoma cell lines, more studies have made notable advancements. One study revealed that for establishing a routine method for isolation of primary horse melanoma cells, surgically excised tissues from 13 grey horses with melanomas were used (Chapman et al., 2009). Their approach involved mechanically mincing the tissues with a scalpel blade and subsequently subjecting the obtained pieces through enzymatic digestion with collagenase. Afterwards, the resulting melanoma cell suspension has been filtered through nylon cell strainers to further separate the cells. In order to confirm the melanoma origin of the obtained equine cell cultures, these researchers have proven the presence of melanoma-associated antigens (Melan-A, MAGE-1 - melanoma-associated antigen 1, MAGE-3 - melanoma-associated antigen 3) (Chapman et al., 2009). Other conducted studies on this topic revealed that the process of trimming the epidermis and the surrounding connective and adipose tissue is favourable in order to obtain pure melanoma cells in the culture (Metzger et al., 2007).

Briefly, most protocols for obtaining equine melanoma cell cultures use enzymatic digestion, being also mentioned mixed solutions with collagenase, hyaluronidase and pronase.

Nevertheless, in 2014, Seltenhammer and her collaborators established for the first time both a primary (HoMel-L1) and a metastatic equine melanoma (HoMeL-A1) cell line only by mechanical mincing the excised tissues and washing the obtained pieces several times in phosphate-buffered saline solution to remove the excessive melanin pigment.

In swine pathology, spontaneous melanoma is rarely encountered, yet it has been occasionally observed in pigmented pig breeds (e.g. Duroc) (Horak et al., 2019).

Due to skin depigmentation in their lifetime and the presence of more actively cytotoxic CD8+ T lymphocytes in tumour microenvironment, more than 85% of swine melanomas can spontaneously regress (Horak et al., 2019).

Additional studies demonstrated that cutaneous melanoma in dark skin miniature breed pigs (e.g. Sinclair, Munich Miniature Swine Troll) have similar histopathological characteristics with human tumours (Aktary et al., 2019; Green et al., 1992).

A pig model for studying hereditary melanoma has been developed in Czech Republic, known as Melanoma-Bearing Libechov minipig (Horak et al., 2019). Further genetical studies on this pig model has demonstrated overexpression of MC1R (melanocortin 1 receptor), RACK1 (receptor for activated C kinase 1), MITF (microphthalmia-associated transcription factor) and KIT genes which has been also found to be implied in human melanoma development. Although swine melanoma cells might represent a valuable model for conducting studies about tumour immune response, due their numerous physiological similarities within species and their tendency of undergoing spontaneous regression, pig melanoma cell lines have been lastly mentioned in articles from 1979 and 1992 (Green et al., 1992; Berkelhammer et al., 1979).

The most encountered variants of melanomas in animals, along with their molecular characteristics and translational potential, are summarized in Table 1. These similarities between animal variants and human melanoma promote future research, highlighting the potential use of animal models in translational studies and the further development and establishment of more animal melanoma cell cultures.

Table 1. Comparative molecular features of principal melanoma variants across species

Species	Variant	Spontaneous occurrence	Genetic mutations	Translational potential for further research
Murine (B16)	Cutaneous Uveal	Yes (genetically engineered)	Induced BRAF, NRAS, HRAS	Drug testing Progression and tumour microenvironment
Canine	Oral/mucosal Acral Uveal Cutaneous	Yes	BRAF, NRAS, KRAS, PTEN, KIT	Further therapy improvement (particularly for mucosal, acral and uveal variants)
Feline	Uveal Cutaneous	Yes	BRAF (rare)	Progression and therapy of uveal melanoma
Equine	Dermal	Yes	NRAS, TP53, KIT, BRAF, KRAS	Therapy improvement
Swine	Cutaneous	Yes, and spontaneous regression	MC1R, RACK1, MITF, KIT	Tumour microenvironment and immune response

CONCLUSIONS

Variants of animal melanoma cell cultures have proven significant contribution for understanding the complexity of pathological and molecular insights of melanoma biology. Thus, these in vitro models facilitated the understanding of signalling pathways (e.g. MAPK/Akt), principal proto-oncogenes mutations and their inhibitors involved in melanomagenesis. Additionally, cross-species studies of melanoma tumours have been improving diagnostic tests and therapeutic strategies in both veterinary and human oncology.

While murine cells remain pivotal for preclinical testing, they do have limitation regarding genetic particularities. Therefore, canine, equine and swine *in vitro* models can enhance the further development of targeted therapies involving the tumour microenvironment. Moreover, swine melanoma spontaneous regression can provide supplementary insights regarding the tumour microenvironment and immune response that can be later translated into specific immunotherapy for all species.

The present review also highlights that feline melanoma is not yet extensively studied, further research is needed in order to better understand the molecular characteristics of melanoma in this species and to develop permanent feline melanoma cell lines.

Lastly, standardization of culturing protocols and further development of three-dimensional cultures and organoids can contribute effect-tively in advancing studies of tumour microenvironment composition, molecular changes and pathological insights involved in melanoma evolution.

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MISCELLANEOUS