expression around TAMs is related to M1 phenotype macrophages, which produce proinflammatory cytokines and help kill tumor cells. Therefore, obesity can affect tumor development by recruiting and polarizing macrophages, and overweight or obese dogs are expected to have a worse prognosis compared with that of lean or normal dogs.

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O-019

Expression of ER-α, ER-β in benign and malignant canine hepatoid gland tumors

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Introduction: The hepatoid gland tumor, also known as perianal gland tumor, is one of the most common skin tumor in aged dogs, but its molecular aspects are not well-defined. The hepatoid gland tumor is known to be affected by the steroid hormone, androgen. Additionally, a recent study has revealed that the hyperplastic and neoplastic canine hepatoid gland does not express tumor suppressive transcription factor, p53. The objective of this study is to analyze the influences of estrogen receptor-α (ER-α), estrogen receptor-β (ER-β) and progesterone receptor (PR) on canine hepatoid gland adenoma and carcinoma. We also investigated the expression of p63, the structural and functional homolog of p53.

Materials and Methods: Total 64 hepatoid gland tumors were carefully screened and classified as adenoma (n=34) or carcinoma (n=30) on H&E sections. The expression of ER-α, ER-β, PR, p63 was analyzed by immunohistochemistry. ER-α expression scores were based on number of positive cells and intensity grade. ER-β, PR, p63 expression was scored based upon number of positive cells. Pearson chi-square test was performed to evaluate the relevance between tumor grade and ER-α, ER-β, PR, p63 expression.

Results: Expression of ER-α was higher in adenomas than in its malignant counterparts (P=0.014). ER-β also showed a significantly higher expression in adenomas than in carcinomas (P=0.000). No significant association was observed between histological classification and p63, PR expression.

Conclusions: Androgen is known to have an proliferative effect on canine hepatoid gland tumors, and estrogen interferes androgen production by decreasing the hypothalamic-pituitary-gonadal axis. As such, canine hepatoid gland tumors might be affected by estrogen and its receptors, ER-α and ER-β. Moreover, following the results, ER-α and ER-β may be a prognostic factor of canine hepatoid tumors. Further study is needed to identify the correlation between estrogen and these tumors.

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O-020

Activation of PI3K/Akt and MEK/ERK signaling pathways facilitates porcine sapovirus trafficking from early to late endosome

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Introduction: Caliciviruses in the genera *Norovirus* and *Sapovirus* are important acute gastroenteritis pathogens in humans and animals. Porcine sapovirus (PSaV) Cowden strain, the only cultivable member in the genus *Sapovirus*, provides a suitable system that can be used to study the life cycle of caliciviruses[1]. Although the PI3K/Akt and MEK/ERK signaling pathways are known to mediate virus entry processes such as viral trafficking or endosomal acidification [2, 3], their role(s)in PSaV entry remains largely unknown.

Materials and Methods: PSaV Cowden strain cultured in LLC-PK cells was used in this study. The phosphorylation of PI3K, Akt and ERK signaling molecules was detected by Western blot at different time points after infection of PSaV Cowden strain, with or without bile acid, as well as its recombinant viral-like particles (VLP). The effect of sodium periodate (NaIO4) and neuraminidase (NA) on Alexa Fluor 594 (AF-594)-labelled PSaV binding to cell surface receptor was detected by confocal microscopy and their influence on activation of PI3K/Akt and MEK/ERK signaling pathways after PSaV infection was checked by Western blot. The colocalization of AF-594-labelled PSaV with early endosome marker EEA1 and late endosome marker LAMP2 was detected using confocal microscopy. The effect of PI3K (wortmannin) and MEK (U0126) inhibitors on entry of AF-594-labelled PSaV was investigated by confocal microscopy. The possible interaction between PSaV-activated signaling molecules and V-ATPase proton pump was determined by immunoprecipitation (IP) assay.

Results: PSaV Cowden strain activated PI3K/Akt and MEK/ERK signaling pathways at immediate early stage of virus infection. Addition of PSaV VLP induced early activation of PI3K/Akt and MEK/ERK signaling pathways. Furthermore, inhibition of PSaV binding to cell surface

receptors by pretreatment of $NaIO_4$ or NA reduced their activation, indicating that PSaV-binding to the cell surface receptors could trigger activation of both signaling pathways. We also presented that PSaV was first transported to early endosomes and eventually to late endosomes, where virus uncoating would take place. Inhibition of PI3K/Akt and MEK/ERK pathways by wortmannin and U0126 resulted in blockage of PSaV particles in early endosomes and prevented their trafficking to late endosomes. Both cascades did not involve in endosomal acidification during PSaV entry, since no significant interaction was seen between these signaling molecules and the V-ATPase following PSaV infection.

Conclusions: PSaV Cowden strain could hijack both cellular PI3K/Akt and MEK/ERK signaling pathways for PSaV trafficking from early to late endosomes.

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O-021

H type and sialyl-Lewis A type HBGAs act as attachment factors for bovine nebovirus entry

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Introduction: Caliciviruses are important veterinary and human pathogens which are associated with a broad spectrum of diseases in their respective hosts [1]. Currently, Caliciviridae family is officially divided into five genera; Norovirus, Sapovirus, Nebovirus, Lagovirus and Vesivirus, and former three genera cause diarrhea in humans and animals. Many caliciviruses enter the cells through binding of cell surface histo-blood group antigens (HBGAs) or terminal sialic acids (SAs)[2]; majority in Norovirus and Lagovirus genera recognizes HBGAs as receptors [3], whereas feline calicivirus, murine norovirus and porcine sapovirus use SAs as receptors. However, it remains largely unknown about bovine nebovirus (BNeV) receptors. Therefore, the aim of this study is to elucidate which cell surface carbohydrate moieties are utilized as an attachment factor for BNeV.

Materials and Methods: Since there is no permissive cell culture system for BNeV, recombinant BNeV-like particle (VLP) produced using baculovirus expression system, and recombinant P domain of the BNeV capsid expressed using E.coli system were employed. To characterize the attachment factor(s) of BNeV, we undertook a comprehensive series of assays including treating cells with a carbohydrate-destroying chemical (NaIO4) or linkage-specific neuraminidase, synthetic HBGA binding assay, and binding assay in CHO cells transfected with each HBGA. Binding affinity of Alexa Fluor 594 (AF-594)- or radioisotope (RI)-labelled BNeV was determined by confocal microscope or beta-counter.

Results: Pretreatment of MDBK cells with 1 mM NaIO4 increased binding affinity of AF594-labelled P domain or RI-labelled VLP, whereas 10 mM NaIO4 markedly decreased binding affinity of AF-594-labelled P domain or RI-labelled VLP, indicating HBGAs but not terminal SAs could be used as attachment factors for BNeV infection. The synthetic HBGA binding assay showed that BNeV VLP significantly bound to H type and sialyl-Lewis A type HBGAs. Furthermore, treatment of fucosidase significantly blocked binding of BNeV VLP to sialyl-Lewis A type HBGA. In comparison with A and B type transfected CHO cells, the H type transfected CHO cells were in fact shown to bind stronger with Alexa 594-labelled VLP.

Conclusions: BNeV could recognize H type and sialyl-Lewis A type HBGAs on the cell surface carbohydrates as attachment factors.

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O-022

Requirement for *Porcine Sapovirus* replication; roles of tyrosine residues in the VPg and association of RdRp with other viral proteins

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