Autocrine control of mammary epithelial cell function

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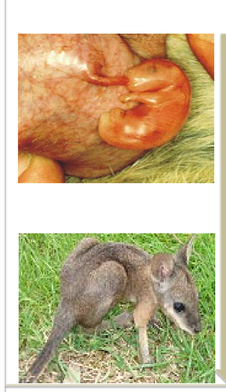
Background

Milk is mainly known for its role in nutrition but there is increasing evidence to show that bioactive molecules in milk may modulate mammary epithelial cell function. This study exploits the unusual reproductive strategy of the tammar wallaby to investigate cathelicidins, MaeuCath1 and 7 secreted in milk for their role in the autocrine control of the mammary gland and antimicrobial defence of the neonate. Both Maeucath 1 and Maeucath7 were found to enhance mammary epithelial cell (WallMEC) proliferation and their derivative peptides Con73 and Con218 showed antibacterial activity. We also show that Maeucath1 is temporally regulated through alternate splicing.

Wallaby lactation

Consists of three post parturition phases (P2A, P2B and P3), each marked by profound changes in milk composition

Wallaby vs human reproductive strategy



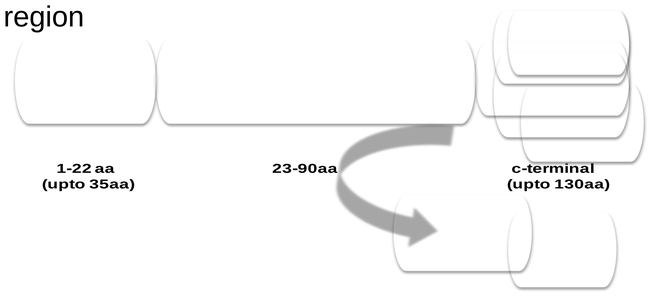
a. short gestation
b. relatively long lactation
c. Greater investment in development during lactation compared to eutherians
d. Mik volume and composition changes

a. long gestation
b. relatively short lactation
c. Greater investment in development during gestation
d. Post-colostoral milk composition does not change significantly

Cathelicidin primary structure

Cathelicidins consist of an N-terminal signal peptide sequence a middle cathelin-like domain and a variable cationic C-terminal sequence. Peptides Con73 and Con218 were designed from the antimicrobial region

MaeuCath1 and 7 respectively.



Methods

Cell proliferation

- MaeuCath1 and 7 were cloned in pTarget® expression vector including a Flag affinity tag
- Protein was synthesized in HEK293 cells and purified using anti-Flag resin
- Purified protein was added to WallMEC growth media for cell culture and cell proliferation determined using the SRB assay

Antimicrobial assay

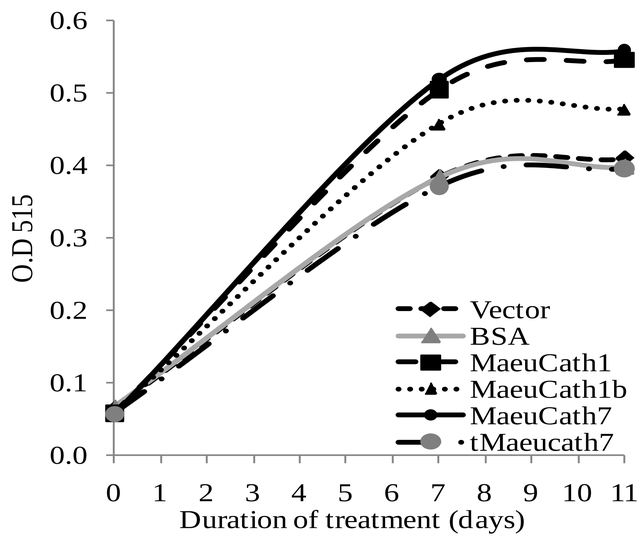
Two linear peptides, Con73 and Con218, derived from the heterogeneous carboxyl end of cathelicidin transcripts, MaeuCath1 and MaeuCath7 respectively, were evaluated for antimicrobial activity.

Temporal regulation of maeucath1

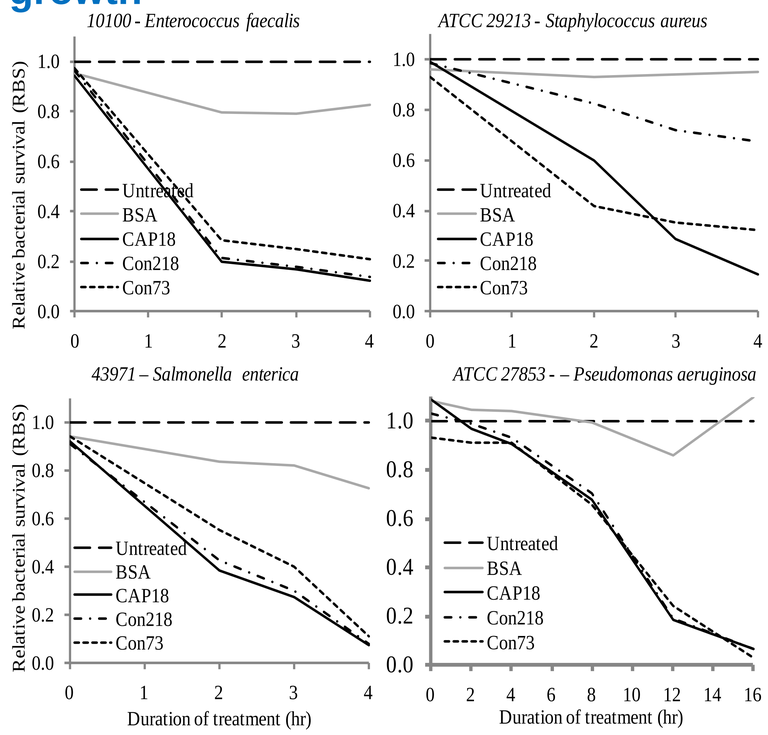
Mammary cDNA prepared from all the phases of lactation and RT-PCR to determine temporal regulation of Maeucath1

Results

MaeuCaths enhance WallMEC proliferation



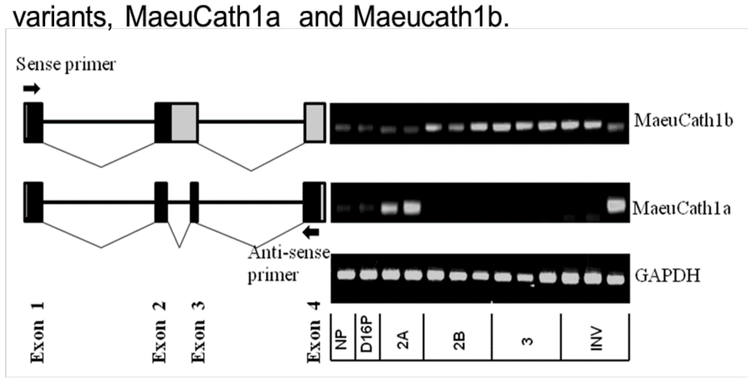
Con73 and Con218 inhibit bacterial growth



Relative bacterial survival (on the Y- axis) was calculated as a fraction of the untreated control.

Alternate splicing of MaeuCath1

RT-PCR on tammar mammary gland cDNA revealed lactation-phase dependent differential splicing into two variants, MaeuCath1a and Maeucath1b.



MaeuCath1a has all 4 exons and has antibacterial activity
MaeuCath1b has cathelin-like region and enhances cell proliferation

Conclusion

Using the reproductive strategy of the tammar wallaby this study shows that the cathelicidin gene is temporally regulated by alternate splicing to provide protection for both the pouch young and mammary gland at the time of increased risk of infection and the proliferation of the mammary gland during the time of increased growth of this tissue.

References

Wanyonyi SS, Sharp JA, Khalil E, Lefevre C, Nicholas KR: Tammar wallaby mammary cathelicidins are differentially expressed during lactation and exhibit antimicrobial and cell proliferative activity. *Comp Biochem Physiol A Mol Integr Physiol*, 2011, 160(3):431-439.