Ratiometric activatable cell penetrating peptide (RACPP) detects malignant lesions in carcinogen-induced model of head and neck squamous cell carcinoma.

Dina V. Hingorani PhD1, Joseph R. Acevedo BS1, Aaron Lemieux MD1, Suraj Kedarissetty MD1, Courtni Salinas BS1, Michael A. Whitney PhD2, Alfredo Molinolo, MD2, Roger Y. Tsien, PhD2, Quyen T. Nguyen, MD/PhD1,2,3

1Division of Otolaryngology, Head and Neck Surgery, 2Department of Pharmacology, 3Moores Cancer Center, University of California San Diego, CA, USA

Abstract

Objective: (a) Detection of spontaneous oral cancer lesions with matrix metalloproteinase (MMP) cleavable ratiometric activatable cell penetrating peptide (RACPP). (b) Demonstrate improved detection of oral lesions that will progress to frank cancer by ratiometric fluorescence compared to examination under white light.

Background and methods: RACPPs are hairpin shaped undergoes proteolysis by endopeptidases, causing a measurable change in the ratio of Cy5:CY7 which is concentration independent and purely based in enzyme activity and can be used for image guided surgery (1). This study shows the efficacy RACPP to detect MMP activity in a spontaneous oral carcinoma mouse model, which is more representative of molecular mechanisms as seen in human disease. A cohort of 14 C57Bl6 mice were given drinking water with 50 µg/ml of carcinogen 4-nitroquinoline oxide (4NQO) for 16 weeks followed by normal drinking water up to week 21 (2). The mice were imaged with PlGmAg RACPP (3) (10 nmol, 100 µl, i.v., 90 min circulation) every alternate week from week 7 to 21 using our in-house surgical microscope under white light and fluorescent light and to generate Cy5:CY7 ratio overlays.

Results: By week 21, 13 of the 14 mice had oral cavity lesions. Cy5:CY7 ratio identifies 1.57 tumors per mouse (ratio intensity range=1 to 29%) which is comparable to 1.50 obtained by white light alone. In a few cases, ratiometric signal identified tumors inconspicuous by white light.

Conclusions: Cy5:CY7 ratio increase is indicative of MMP catalyzed cleavage of RACPP and co-localizes with lesions that are not always readily identifiable as cancerous under white light making it a diagnostic tool for confirmation of cancer non-invasively.

 Imaging Technology

Ratiometric activatable cell penetrating peptides (RACPPs) are hairpin like molecular probes that undergo a measurable change in the ratio of Cy5:CY7 fluorescence intensity upon cleavage by the enzyme and a fragment of which is retained at the site of activation (A). RACPP contain two short polypeptides made of d-arginine and d-glutamine are held together by an enzyme cleavable short peptide. The polypeptide made of d-arginine has a Cy5 fluorophore while the polypeptide of d-glutamine has the Cy7 fluorophore on its end. The close proximity of these fluorophores causes the Cy5 signal to be quenched by Cy7. Upon separation of the two chains by cleavage by the enzyme the Cy5 fluorescence is enhanced and the polycarbonate chain adheres to the negatively charged cell membrane for internalization (3). In an orthotopic xenograft mouse tongue tumor model of CaI-27 cells in Athymic Nu/Nu mice (B) RACPP injection produces greater ratiometric fluorescent signal in HN5C5 tumor versus normal tissue (C). Un cleavable-control does not produce tumor-specific contrast (D) (Figure adapted from Haufl et al 2014; 4).

 Experimental Design

Cohort 1: A cohort of 14 mice were given 4NQO in their drinking water for 16 weeks at which time they were converted to regular drinking water until week 21. White light and ratiometric fluorescent (RF) imaging was done biweekly starting at 11 weeks and continuing until week 21 at which time tissue was collected.

Cohort 2: A cohort of 4 mice were given 4NQO in their drinking water for 16 weeks followed by white light and RF imaging with endpoint tissue collection.

 Results

Tumor progression in cohort 1 (n=14). Number of tumors of per animal and the tumor size distribution shown as a function of time*. (A), tumors visible with white light reflectance. (B), tumors visible with Cy5:CY7 ratiometric fluorescence. The tumors per mouse visible in white light and under RF are nearly identical each week, but the size distribution varies.

*Lesions that did not persist until week 21 were excluded.

Conclusions

1. Previously, we have shown the efficacy of MMP cleavable Ratiometric Activatable Cell Penetrating Peptide for detecting orthotopic oral tumors in xenograft mouse models. Here we have shown the ability of RACPP to detect preneoplastic and malignant lesions in a carcinogen induced tumor model in immunocompetent mice.

2. With a tumor to background cut off 0.5 we are non-invasively able to detect oral cavity squamous cell carcinoma over background normal tongue with a sensitivity of 89% and a specificity of 100%.

3. However, MMP responsive RACPPs cannot distinguish between benign and malignant lesions in these immunocompetent mice as it is known that all benign lesion will become malignant in these mice.

4. This study is a proof of concept work where RACPPs maybe used as a non-invasive screening tool for high risk patients in the clinic.

Acknowledgments

This research was sponsored by the National Institutes of Health, Grant No. NIH (NIDR)

R01 EB001402-01

Joseph Acevedo was funded by NIH #1T32. TR001443. Special thanks to Susan Johnson, Paul Steinbach and Qing Kong for technical assistance.


