I. Nisha Jacinta D'Silva

Donald A Kerr Endowed Collegiate Professor of Oral Pathology,
Associate Chair, Division of Oral Medicine, Pathology, Radiology,
Associate Professor, Department of Periodontics and Oral Medicine, School of Dentistry
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II. Education and Advanced Training

2009	Certificate in MBA Essentials and Entrepreneurship.	University of Michigan, Ross School of Business.
1998	Certificate – treatment of disabled patients (DECOD)	University of Washington, Seattle.
1990-1997	PhD	University of Washington, Seattle.
1987-1990	MSD and Residency in Oral and Maxillofacial Pathology	Indiana University, Indianapolis
1983-1987	BDS (DDS)	University of Bombay, India
1981-1983	HSC (Physics, Chemistry, Biology, Mathematics)	Sophia College, Maharashtra State Board, India

III. Academic appointments

09/2009 - present	Associate Professor, Department of Pathology, University of Michigan Medical School.
3/2008 – present	Associate Director, Division of Oral Medicine, Pathology, Radiology
9/2007 – present	Donald Kerr Endowed Collegiate Professor of Oral Pathology.
9/2007 – present	Associate Professor with tenure, Department of Periodontics and Oral Medicine, University of Michigan.
02/2006 – 08/2009	Assistant Professor, Department of Pathology, Medical School.
05/2004 – 02/2008	Director, Oral and Maxillofacial Pathology Biopsy Service, University of Michigan.
05/2003-08/2003	Interim Director, Oral and Maxillofacial Pathology Biopsy Service,
05/2002-08/2002	Interim Director, Oral and Maxillofacial Pathology Biopsy Service.
10/2002 – 08/2007	Director, Head and Neck Cancer Tissue Core, University of Michigan Cancer Center.
05/2001-08/2001	Interim Director, Oral and Maxillofacial Pathology Biopsy Service.
05/2000-08/2000	Interim Director, Oral and Maxillofacial Pathology Biopsy Service.
08/1999 – 04/2000	Clinic Attending, Oral Medicine Clinic, University of Michigan
10/1998 – 8/2007	** Assistant Professor, Department of Oral Medicine, Pathology, Oncology, University of Michigan.
01/1994- 06/1998	Acting Instructor, Department of Oral Biology, University of Washington.
07/1987-06/1990	Associate Instructor, General Pathology Laboratory, Indiana University.

** Department merged with Periodontics and Oral Medicine in September, 2005.

IV. Certificates and licenses

1999-present	Dental License, State of Michigan.
1997-present	Dental License, State of Washington.
1993 - present	Diplomate of the American Board of Oral and Maxillofacial Pathology.
1992 - present	Fellow of the American Academy of Oral and Maxillofacial Pathology.
1987- 1990	Certificate in Oral and Maxillofacial Pathology.

V. Honors and professional awards

2010- 2011	Selected for Executive Leadership in Academic Medicine program (ELAM).
9/2007 – present	Donald Kerr Endowed collegiate professor, University of Michigan.
2006	Crosby Award (NSF-funded ADVANCE program) for women in science.
2005-2006	Selected for American Dental Education Association leadership institute.
2004	Selected for faculty membership of Omicron Kappa Upsilon – Chi Chapter, dental honorary society, on the basis of outstanding contributions to dentistry
2001, 2002	Special Dean's Award for Research, University of Michigan
1996	IADR Hatton award winner, Post-doctoral category
1996	AADR Hatton award winner; Post-doctoral category
1993-1994	Warren Magnuson Scholarship for academic excellence and potential for contribution to research, University of Washington.
1987	J.N. Tata Scholarship, (National award - India).
1987	J. R. D. Sethna Scholarship, (City award – Bombay).
1987	Shri Brihad Bharatiya Samaj Scholarship.
1983 – 1987	Sir Dorab Tata Scholarship for excellence in academic and extra-curricular activities (City award – Bombay). Awarded annually.
1983-1984	St. Luke's Medical Guild Scholarship.
1982-1983	College Union Committee Scholarship for the best All-Round Student of Sophia (Junior) College.

VI. Memberships and offices in professional and research societies (to present)

2011 –	ELAM Alumni association
2010 –	Michigan Center for Translational Pathology
2001 –	American Association for Cancer Research
2001 –	Oral Health Sciences Training Program, University of Michigan
2000 –	University of Michigan Cancer Center
1994 –	AADR/ International Association of Dental Research.
1992 -	Fellow, American Academy of Oral and Maxillofacial Pathology
1993 -	Diplomate, American Board of Oral and Maxillofacial Pathology
1999 –	Head and Neck Oncology program, University of Michigan Cancer Center
2011 - present	Co-Chair, Hatton Awards Committee, American Association of Dental Research
2010 - 2011	Member, Hatton Awards Committee, American Association of Dental Research
2008 - present	Member, External Advisory Committtee, South Carolina COBRE grant for Oral Health Research.
2006 - 2007	Member, Nominations Committee, American Academy of Oral and Maxillofacial Pathology.
2005 - 2006	Member, Inter-HN SPORE (head and neck Specialized Program of Research Excellence) Pathology Working Group.

VII. Teaching activities

Course Director *(*course lecturer/ instructor not included)
(F= Fall, W= Winter, Sp = Spring, Su = Summer)

2011 - present	*DENT605 Neoplasia.*
Sp, Su	*Developed new course for sophomore dental students.*
2012 -	*ORALPATH 602. Oral Pathology Literature Review.*
W	*New course for Oral Pathology Residents.*
2008 – present F, W, Sp, Su	*ORALHEAL 995 Dissertation Research*
2010 – present (co-mentor)	*MCDB300 Undergraduate research.*
F, W, Sp.	*Students perform their research projects in my laboratory.*
2001 – 2011;	*DH393: General and Oral Pathology for Dental Hygiene Students.*
*W**	*Restructured course, introduced clinical case presentations.*
2012; (Co-director) W	*DH393: General and Oral Pathology for Dental Hygiene Students.*
2008 - 2009 W, Sp, Su, F	*ORALHEAL 990 Dissertation Pre-candidate Research*
2008 W, Sp	*ORALHEAL 812 Science Lab Rotation*
2007 W	*ORALHEAL 995 Dissertation Research*
2004 – 2007 (Co-director)	*DENTED 614: Clinical Oral Pathology for Dental Residents.*
W	*Developed new course for Residents in dental school.*
2001- 2008 (Co-director)	*OP625: Sophomore Oral Pathology Laboratory for Dental Students.*
Winter	*Online case-based laboratory with interactive workbook and virtual microscope.*

** During Winter 2009, I was on sabbatical and did not teach the DH393 course.*

Thesis Advisor

2009 –	*Christie Springstead-Scanlon*, DDS-PhD student.
	PhD mentor. Successful NIH F30 grant application.
2007-	*Elizabeth van Tubergen*, DDS.
	PhD mentor. Successful NIH F32 and NIH Loan repayment grant applications.
2001-2006	*Bradley Henson*, DDS.
	PhD mentor. Successful NIH K08 and NIH Loan repayment grant applications.
	Currently Assistant Professor at Western University of Health Sciences, CA.
2011 -	*Ronald Inglehart*, undergraduate student. Honors thesis mentor.

Graduate (PhD) Student Research Rotations

2008	*Christina Springstead-Scanlon*, DDS/PhD student.
2008	*Andres Quintero Valencia*.
2007	*Elizabeth van Tubergen*.
2006	*Keith Dobracki,* DDS/PhD student.
2003	*Joanna Hooten*.
2001	*Elisabeta Karl*.
2000	*Bradley Henson*.

Dental Students: Elective Rotation/ Summer Fellowships/ Mentorship

2011	*Robert Vander Broek*, AADR fellowship.
2010	*Robert Vander Broek*, AADR fellowship. Presented research at national meeting, published manuscript.
2009	*Robert Vander Broek*, Fellowship.
2008	*Carol Kirkpatrick*. Published manuscript.
2009	*Josh van Horn*.
2007	*Stacey Griffith*. Published manuscript
2006	*Sonia Palit*.
2003	*Sam Meyrowitz*, Published manuscript.
2003	*Joanna Hooten*.
2001	*Shirley Paek*. Published manuscript.

Undergraduate Student Research Rotations (since 2005)

2010 –	*Sharvil Shah*.
2009 –	*Tarek Metwaly*.
2009 –	*Joel Lints*.
2009 –	*Ronald Inglehart*.
2011 summer	*Alison Smith*, Kalamazoo College, Michigan.
2009- 2010	*Christine Convery*.
2009- 2010	*Jeffrey Budzyn*.
2009- 2010	*Cyril Mazhuvanchery*.
2005-2007	*Diana Maldonado*.

Medical School Resident Research Rotations

2003 – 2005	*Keith Wolter*, MD, Plastic Surgery Fellow.
2002- 2003	*Steven Wang*, M.D., Otolaryngology/Head and Neck Surgery Fellow. Currently Assistant Professor at UCSF.
2002	*Brandon Isaacson*, M.D., Otolaryngology/Head and Neck Surgery Fellow.

Post-doctoral Fellows

2008 - present	*Rajat Banerjee*, PhD.
2005 – 2008	*Mitsuo Goto,* DDS, PhD.
2004 – 2005	*Malini Pandarpurkar*, PhD.
2001 – 2005	*Zhaocheng Zhang,* MD, PhD.

Awards received by students mentored

2011	*Robert Vander Broek*, AADR fellowship.
2011	*Robert Vander Broek*, oral presentation and dental student travel award, American Academy of Oral and Maxillofacial Pathology (AAOMP) meeting, Puerto Rico
2011	*Elizabeth van Tubergen*, 1st place, post-doctoral category, Research day.
2010	*Robert Vander Broek*, AADR fellowship.
2010	*Robert Vander Broek*, dental student travel award, AAOMP meeting, Phoenix, AZ.
2010	*Elizabeth van Tubergen*, 3rd place, post-doctoral category, Research day.
2010	*Robert Vander Broek*, 1st place, pre-doctoral category, Research Day.
2005	*Bradley Henson*, Dziewiatkowski award, School of Dentistry.
2005	*Bradley Henson*, Gorlin award for excellence in research, AAOMP.
2005	*Bradley Henson*, DDS, Hatton award, Post-doc category, AADR.

2003	*Bradley Henson*, DDS. 1st prize, Post-doc/ Staff category, Research Day.
2003	*Suprotim Samaddar*, Kalamazoo College, Michigan. Research presentation, AADR, San Antonio.
2002	*Bradley Henson*, DDS. 1st prize, Post-doc category, Research Day.
2002	*Shirley Paek*, Dental Student, 3rd prize, Current Topics, Research Day.

PhD degree Thesis Committees

2010 -	Chair. *Christine Springstead-Scanlon*
2008 - 2012	Member. *Turki Alhazzazi*.
2007-	Chair. *Elizabeth van Tubergen*.
2003 – 2006	Chair. *Bradley Henson*.
2002 – 2004	Member. *Abraham Schneider*

Master's degree thesis committee

2007 – 2009	Member. *Suncica Travan*.

VIII. Service

Editorial Boards (current)

2009 -	Head and Neck Pathology,
2008 - 2011	Journal of Dental Research,.
2007 –	Oral Surg, Oral Med, Oral Path, Oral Radiol, and Endodontics.

Journal Reviewer (selected from over 20)

2011 -	Oncogene
2011 –	Carcinogenesis
2011 -	Genes, Chromosomes and Cancer
2011 -	PLoS One
2010 –	Clinical Cancer Research
2010 –	International Journal of Cancer
2010 –	Molecular and Cellular Biology
2010 –	Journal of Oral Pathology
2009 –	British Journal of Cancer
2009 –	Cell Cycle
2008 –	Cancer Research
2008 –	Molecular Cancer Research
2008 –	Molecular Cancer Therapeutics
2008 –	Annals of Surgical Oncology
2004 –	Neoplasia

Grant Reviewer (2007 – present)

2010 – 2016	Special Grants Review Committee, NIDCR/NIH
2010	Cancer Research, United Kingdom.
2010	US-Israel Binational Science Foundation, Israel.
2008	Medical Research Council, United Kingdom.
2008 -	Medical University of South Carolina.
2008	University of Pennsylvania, School of Medicine.

Clinical and Patient Care

1999 – present	Pathologist, Oral Pathology Biopsy Service (~3000 cases/ year).
1999 – 2000	Clinic Attending, Dental Clinics, University of Michigan.

IX. Invited research seminars or lectures at other institutions, meetings or conferences (since 2000)

November 2011	Seminar: "Biomarkers in head and neck cancer: Rap1 and its regulatory proteins". Cancer Center, Lousiana State University.
November 2011	Grand Rounds: "Cysts and Tumors of the Jaws". Lousiana State University Health Sciences Center.
June 2011	Seminar: "Rap1 in head and neck cancer: Role in tumor progression". University of Connecticut Dental Dean's seminar.
April 2011	Presentation: "Development of an Instrument to Evaluate Faculty Members' Annual Performance in a School of Dentistry". ELAM Institutional Action Projects Symposium for Fellows, Deans and Chief Officers of Academic Institutions.
March 2011	Session Chair: "Oral Carcinogenesis" AADR meeting, San Diego, CA.
November 2010	Presentation: Investigation of the "Humoral Signature" in saliva from head and neck cancer patients. HN SPORE External Advisory Committee.
October 2010	Invited presentation: "Biomarkers in head and neck cancer progression". American Head and Neck Society Research Workshop on the Biology, Prevention and Treatment of Head and Neck Cancer. Arlington, VA.
June 2010	Seminar: "The Role of Small GTP-binding proteins in Head and Neck Cancer Progression", University of Michigan.
May 2010	Clinico-pathologic conference case presentation, AAOMP meeting, Tuscon, AZ.
May 2010	Seminar: "The Role of Rap1 in Head and Neck Cancer Progression", University of Michigan Medical School.
March 2010	Session Chair: Oral Cancer I and HIV; Cell Signaling and Differentiation. AADR meeting, Washington DC.
February 2010	Seminar: "Oral cancer: Detection and diagnosis." Nair Hospital Dental College, University of Bombay, India.
February 2010	Seminar: "Rap1 in oral cancer: A translational journey." Nair Hospital Dental College, University of Bombay, India.
April 2009	Session Chair: "Oral Carcinogenesis." AADR meeting, Miami, FL.
April 2008	Session Chair: "Oral Cancer." AADR meeting, Dallas, TX.
September 2008	Seminar: "Rap1 in oral cancer: Mechanistic and functional significance." Medical University of South Carolina.
May 2007	Seminar: "Oral cancer: Growth, invasion and survival." National Institutes of Health.

April 2007	Seminar: "Rap1-mediated Growth and Invasion in Oral Cancer." University of California, Los Angeles.
November 2006	Seminar: "Oral Cancer: A Story of Growth, Spread and Survival." New York University.
2006	Session Chair: "Oral Cancer: Factors Affecting Growth and Metastasis." AADR meeting, Orlando, FL.
September 2006	Seminar: "Oral Cancer: A Tale of Growth and Survival." University of Florida.
March, 2004	Seminar: "Rap1: Role in Oral Cancer" Ottawa Regional Cancer Center, Canada.
January, 2002	Malignant Salivary Gland Tumors and Clinico-pathologic conference Oral Surgery Residents, Henry Ford Hospital.
September, 2001	Malignant Oral Epithelial Lesions and Clinico-pathologic conference. Oral Surgery Residents, Henry Ford Hospital.
August, 2001	Benign and Premalignant Oral Epithelial Lesions. Oral Surgery Residents, Henry Ford Hospital.

X. Grant support (Current)

Project Name: Role of rap1 in cadherin and integrin-mediated cross-talk in oral keratinocytes.
Funding Agency: NIH/NIDCR R01 DE018512
Principal Investigator: Nisha D'Silva; 20% effort
Total grant: $1,710,000.
Project period: 8/1/07 – 7/31/2012

Project Name: Biomarkers in head and neck cancer progression. (Independent scientist award).
Funding Agency:NIH/NIDCR K02 DE019513
Principal Investigator: Nisha D'Silva; 43% effort.
Total grant: $472,270.
Project period: 4/7/09 – 4/6/14

Project Name: The Molecular Basis of Head and Neck Cancer biology, treatment and prevention.
Funding Agency: NIH NCI NIDCR SPORE 1 P50 CA97248
Principal Investigator: Gregory T. Wolf
Role: Pathologist; 5.5% effort.
Total direct Costs (Year 1): $1,756,908
Project period: Competing renewal funded 7/1/08 – 6/30/13

Project Name: EZH2-mediated invasion in Squamous Cell Carcinoma.
Funding Agency: University of Michigan Comprehensive Cancer Center Pilot grant.
Principal Investigator: Nisha D'Silva;
Total Direct Costs: $40,000 for 1 year.
Project period: 5/1/11 – 4/30/12.

Project Name: The role of tristetraprolin in head and neck cancer progression.
Funding Agency: NIH NIDCR Ruth L. Kirschstein National Research Service Awards (F32).
Principal Investigator: Elizabeth vanTubergen DDS, (PhD candidate in my lab)
Role: Mentor. No salary support requested.
Total Direct Costs: $122,976 for 2 years.
Project period: 6/1/10 – 5/31/12

Project Name: Head and neck cancer: Role of NFATc2 in GALR2-mediated tumor progression.
Funding Agency: NIH NIDCR Ruth L. Kirschstein National Research Service Awards (F30).
Principal Investigator: Springstead-Scanlon, (DDS-PhD candidate in my lab)
Role: Mentor. No salary support requested.
Total (direct and indirect) amount of grant: $94,360 for 2 years.
Project period: 6/1/10 – 5/31/12

XI. Publications

A. Peer reviewed

1. Banerjee R, Mani R, Russo N, Scanlon CS, Tsodikov A, Jing X, Cao Q, Palanisamy N, Metwally T, Inglehart RC, Tomlins S, Bradford C, Carey T, Wolf G, Kalyana-Sundaram S, Chinnaiyan A, Varambally S and **D'Silva** NJ. The tumor suppressor gene rap1GAP is silenced by mir-101-mediated EZH2 overexpression in invasive squamous cell carcinoma. 2011 Oncogene. 20;30(42):4339-49.
2. Banerjee R, Henson, Russo, Tsodikov, **D'Silva** NJ. Rap1 mediates Galanin receptor 2-induced proliferation and survival in squamous cell carcinoma. 2011 Cellular Signalling, 23(7):1110-8.
3. Kamarajan P, Alhazzi T, Danciu T, **D'Silva** NJ, Verdin E, Kapila Y. Receptor interacting protein (RIP) and Sirtuin-3 (SIRT3) are on Opposite Sides of Anoikis Resistance and Tumorigenesis. 2012 Cancer*, In Press*.
4. Wansom D, Light, Thomas, Worden, Prince, Urba, Chepeha, Kumar, Cordell, Eisbruch, Taylor, Moyer, Bradford, **D'Silva** NJ, Carey, McHugh, WolfGT. Tumor Infiltrating Lymphocytes and HPV-16 Associated Oropharynx Cancer. 2011, Laryngoscope. 2012 Jan;122(1):121-7.
5. Van Tubergen E, Vander Broek, Lee, Wolf, Carey, Bradford, Prince, Kirkwood, **D'Silva** NJ. Tristetraprolin regulates IL-6 which is correlated with tumor progression in head and neck squamous cell carcinoma. 2011 Cancer, 117(12):2677-89.
6. ***, ****Murdoch-Kinch CA, Russo, Griffith, Braun, Eisbruch, **D'Silva** NJ. Recovery of Salivary EGF in Parotid Saliva Following Parotid Sparing Radiation Therapy: A Proof of Principle Study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2011 111(1):64-70.
   ***** Highlighted on journal cover.
   ****** Millard Award for Best Paper of the Oral Medicine Section of OOOOE, 2011.
7. Zhao W, Liu, **D'Silva** NJ, Kirkwood. Tristetraprolin Regulates IL-6 Expression Through p38 MAPK Dependent Affinity Changes with mRNA 3'UTR. 2011 Journal of Interferon and Cytokine Research, Aug;31(8):629-37. PMID: 21667401.
8. Arthur AE, Duffy SA, Sanchez, Gruber, Terrell, Hebert, Bradford, **D'Silva** NJ, Carey, Wolf GT, Peterson KE, Rozek LS. Dietary factors associated with HPV-positive head and neck cancer: a case-only analysis. 2011 Nutrition and Cancer: An International Journal, 63(5):734-42.
9. Scheller EL, Kirkpatrick, **D'Silva** NJ, Krebsbach, EdwardsPC. Bisphosphonates inhibit expression of P63 by oral keratinocytes. 2011 Journal of Dental Research, Jul;90(7):894-9. PMID: 21551338.
10. Goto, M, Mitra, Liu, Lee, Henson, Carey, Bradford, Prince, Wang, Fearon, **D'Silva** NJ. Rap1 Stabilizes β-catenin and Enhances β-catenin-dependent Transcription and Invasion in Squamous Cell Carcinoma of the Head and Neck. Clin Cancer Res. 2010; 16(1):65-76.
11. Patel MM, Wilkey, Abdelsayed, **D'Silva** NJ, Malchoff, MallyaSM. Analysis of GNAS mutations in cemento-ossifying fibromas and cemento-osseous dysplasias of the jaws. Oral Surg, Oral Med, Oral Patho, Endo. 2010; 109(5):739-43
12. Stanbery L, **D'Silva** NJ, Lee, Bradford, Carey, Prince, Wolf, Worden, Cordell, PettyEM. High SEPT9_v1 expression is associated with poor clinical outcomes in head and neck squamous cell carcinoma. Translational Oncology, 2010 3(4):239-45.
13. Kamarajan P, Garcia-Pardo, **D'Silva** NJ, KapilaYL. The CS1 segment of fibronectin is involved in human OSCC pathogenesis by mediating OSCC cell spreading, migration, and invasion. BMC Cancer. 2010;10:330.
14. Fowler CB, **D'Silva** NJ. Clinico-Pathologic Conference: Case 5. Head and Neck Pathology, 2010 4(3):234-7.
15. Wansom D, Light, Worden, Prince, Urba, Chepeha, Cordell, Eisbruch, Taylor, **D'Silva** NJ, Moyer,

Bradford, Kurnit, Kumar, Carey, Wolf GT. Cellular Immunity Correlates with HPV-16 Status and Outcome in Patients with Advanced Oropharyngeal Cancer. Archives of Otolaryngol Head and Neck Surg, 2010 136(12):1267-73.

16. Alhazzi T, Kamarajan, Nam, Verdin, **D'Silva** NJ, Kapila Y. Sirtuin-3, A Novel Potential Therapeutic Target for Oral Cancer. 2010 Cancer, *In Press.* PMID: 21117229.
17. Bashutski JD, **D'Silva** NJ, Wang H-L. Implant compression necrosis: Current understanding and case report. J Periodontol. 2009; 80(4):700-4.
18. Chang PC, Cirelli, Jin, Seol, Sugai, **D'Silva** NJ, Chandler, Sosnowski, Giannobile WV. Adenovirus Encoding Human Platelet-Derived Growth Factor-B Delivered to Alveolar Bone Defects Exhibits Safety and Biodistribution Profiles Favorable for Clinical Use. Hum Gene Ther. 2009; 20(5):486-96.
19. Silva FWG, **D'Silva** NJ, Silva, Kapila YL. High MMP Activity is a Hallmark of Periapical Granulomas. J Endod. 2009; 35(9):1234-42.
20. **D'Silva** NJ, Benian, Flint, Cordell. Soft tissue swelling of the upper lip. Oral Surg, Oral Med, Oral Pathol, Oral Radiol, Endod 2008 105(3):271-3.
21. Kumar B, Cordell, Lee, Worden, Prince, Tran, Wolf, Urba, Chepeha, Teknos, Eisbruch, Tsien, Taylor, **D'Silva** NJ, Yang, Kurnit, Bauer, Bradford, Carey T. EGFR, p16, HPV Titer, BCLXL and p53, Gender and Smoking as Indicators of Response to Therapy and Survival in Oropharyngeal Cancer. J Clin Oncol. 2008 1;26(19):3128-37.
22. Worden F, Kumar, Lee, Wolf, Cordell, Taylor, Urba, Eisbruch, Teknos, Chepeha, Prince, Tsien, **D'Silva** NJ, Yang, Kurnit, Mason, Miller, Wallace, Bradford, Carey T. Chemoselection as a Strategy for Organ Preservation in Advanced Oropharynx Cancer: Response and Survival Positively Associated with HPV16 Copy Number. J Clin Oncol. 2008 26(19):3138-46.
23. Cudney N, Persico, Cordell, **D'Silva** NJ. Adenomatoid odontogenic tumor developing in association with an odontoma: Report of a case. Quintessance International, 2008 Sep;39(8):693-7.
24. Chiu E, Havens, Taba, Wang, Sun, Jung, Taichman, **D'Silva** NJ, Gopalakrishnan, Wang, Giannobile, Taichman RS. Stromal Derived Factor-1α Levels Increase During Periodontal Disease. J Perio, 2008 May; 79(5):845-853.
25. Kumar B, Cordell, **D'Silva** NJ, Prince, Adams, Fisher, Wolf, Carey, Bradford C. Expression of p53 and Bcl-xL as Predictive Markers for Larynx Preservation in Advanced Laryngeal Cancer. Arch Otolaryngol Head Neck Surg. 2008 Apr;134(4):363-9.
26. Mitra RS, Goto, Lee, Maldonado, Taylor, Pan, Carey, Bradford, Prince, Cordell, Kirkwood, **D'Silva** NJ. Rap1GAP promotes invasion via induction of MMP-9 secretion, which is associated with poor survival in early N-stage squamous cell carcinoma. Cancer Research 2008 68(10):3959-69.
27. Patil C, Liu, Zhao, Coatney, Li, vanTubergen, **D'Silva** NJ, Kirkwood. Targeting mRNA stability arrests inflammatory bone loss. Molecular Therapy. 2008, 16(10):1657-64.
28. Liu H, Henson, Zhou, **D'Silva** NJ, Mistretta CM. Fungiform papilla pattern: EGF regulates inter - papilla lingual epithelium and decreases papilla number via PI3K/Akt, MEK/ERK and p38 MAPK signaling. Developmental Dynamics. 2008, 237(9):2378-93.
29. Kirkwood KL, Li, Rogers, Otremba, Coatney, Kreider, **D'Silva** NJ, Chakravarty, Dugar, Protter, Medicherla S. A p38alpha selective mitogen-activated protein kinase inhibitor prevents periodontal bone loss. J Pharmacol Exp Ther. 2007 320(1):56-63.
30. Chiou S, Gobetti, **D'Silva** NJ. Mucous Membrane Pemphigoid: A retrospective study. Michigan Dental Journal, 2007, 89:46-52.
31. Henson B, Li, Coatney, Carey, Mitra, Kirkwood, **D'Silva** NJ. An orthotopic floor-of mouth model for loco-regional growth and spread of human squamous cell carcinoma. Journal of Oral Pathology and Medicine 2007, July 36(6): 363-70.
32. Bauer JA, Kumar , Cordell, Prince, Tran, Wolf, Chepeha, Teknos, Wang, Eisbruch, Tsien, Urba, Worden, Lee, Griffith, Taylor, **D'Silva**, Wang, Wolter, Henson, Fisher, Carey, Bradford CR. Targeting Apoptosis to Overcome Cisplatin Resistance: A Translational Study in Head and Neck Cancer. Int. Journal of Radiation Oncology, Biology, Physics, 2007, 69:S106-8.
33. Kumar B , Cordell , Lee, Prince, Tran, Wolf, Urba, Worden, Chepeha, Teknos, Eisbruch, Tsien, Taylor , **D'Silva** NJ, Yang, Kurnit, Bradford, Carey T. Response to Therapy and Outcome in Oropharyngeal

Cancer Are Predicted by Biomarkers Including HPV, EGFR, Gender and Smoking. Int. Journal of Radiation Oncology, Biology, Physics. 2007, 69:S109-11.

34. Cordell KG, Stannard, **D'Silva** NJ. AAOMP case challenge: "Erythematous burning lips". J Contemp Dent Pract. 1;7(2):160, 2006.
35. **D'Silva** NJ, Cordell, Flint, Allen, Machiorlatti, Zietz L. Odontogenic Sarcoma with Smooth Muscle Differentiation: Report of a case and Review of the Literature. Oral Oncology Extra, 2006; 42: 273-276.
36. Zhang L, Chenwei, Mahmood, vanGolen, Greenson, L, **D'Silva** NJ, Li, Burant, Logsdon, Simeone DM. Identification of a putative tumor suppressor gene rap1GAP in pancreatic cancer. Cancer Res. 66(2):898-906, 2006.
37. Zhang Z, Mitra, Henson, Datta, McCauley, Kumar, Lee, Carey, **D'Silva** NJ. Rap1GAP inhibits tumor growth in oropharyngeal squamous cell carcinoma. Am J Pathol. 168(2):585-96, 2006.
38. Wolter KG, Wang, Henson, Wang, Griffith, Kumar, Chen, Carey, Bradford, **D'Silva** NJ. (-)-Gossypol inhibits growth and promotes apoptosis of human head and neck squamous cell carcinoma in vivo. Neoplasia 2006 8(3):163-172.
39. **D'Silva** NJ , Summerlin, Cordell, Abdelsayed, Tomich, Hanks, Fear, Meyrowitz. Metastatic Tumors to the Jaws: Retrospective Study of 114 cases. JADA 2006 Dec;137(12):1667-72.
40. Henson BS, Neubig, Jang, Ogawa, Zhang, Carey, **D'Silva** NJ. Galanin Receptor 1 has anti-proliferative effects in oral squamous cell carcinoma. J Biological Chemistry 280(24):22564-71, 2005.
41. Chen C, Koh, Datta, Jhang, Keller, Xiao, Franceschi, **D'Silva** NJ, McCauley LK. Impact of the Mitogen Activated Protein Kinase Pathway (MAPK) on Parathyroid Hormone Related Protein Actions in Osteoblasts. J Biological Chemistry. 279(28):29121-9, 2004.
42. **D'Silva** NJ; Mitra, Kurnit, Zhang, Babcock, Polverini, Carey: Rap1B, a small GTP-binding protein is upregulated during arrest of proliferation in human keratinocytes. J Cellular Physiol. 196(3) 532-40, 2003.
43. Mitra R, Zhang, Henson, Kurnit, Carey, **D'Silva** NJ: Rap1A and rap1B ras-family proteins are prominently expressed in the nucleus of squamous carcinomas: Nuclear translocation of GTP-bound active form. Oncogene. 22(40): 6243 – 56, 2003.
44. **D'Silva** NJ, Hanks, Paulino, Kurago, Polverini: Intraoral mucosal melanoma in a child: A case report. Arch Pathol Lab Medicine. 126(9):1110-1113, 2002.
45. Chen H, McCauley, **D'Silva** NJ: Cyclic AMP binding protein assay for widespread use in cell signaling studies. Biotechniques 33(1): 66-72, 2002.
46. **D'Silva** NJ, Jacobson, Ott, Watson. Beta-adrenergic-induced cytosolic redistribution of Rap1 in rat parotid acini: role in secretion. American J Physiol. Cell Physiol 274:C1667-C1673, 1998.
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Impact Statement

I have used clinical skills as a pathologist to inform my bench research by asking clinically relevant questions, and to teach. Similarly, I have used my bench skills to investigate clinical observations, and to teach the next generation of clinician-scientists.

The focus of my research is identification of biomarkers of early detection and tumor progression, and novel treatment targets in head and neck cancer (SCCHN). My laboratory is a leader in the field of rap1 biology and its role in SCCHN development and progression. In a series of papers published in high impact oncology journals such as Oncogene, Cancer Research, and Clinical Cancer Research, we showed that rap1 has a critical role in SCCHN and is activated by GalR2, which is oncogenic. We established that rap1 is expressed in the nucleus of cancer cells; a unique finding since only three of over 100 members of its family of ras proteins, translocate to the nucleus. We investigated the regulatory mechanisms for this nuclear translocation and showed that rap1 promotes nuclear translocation of β-catenin, to facilitate gene transcription and tumor progression. Elucidation of the mechanism of nuclear translocation of β-catenin is significant in developing novel strategies to inhibit its effects in multiple cancers.

Importantly, we showed that rap1GAP, a protein that inactivates rap1, suppresses tumor growth but promotes invasion. Taken together these studies supported a role for rap1GAP as a biomarker for small (early stage) but aggressive (invasive) oral cancers. Using archival tissue linked to survival data we observed that rap1GAP-induced metalloproteinase (MMP9) secretion is linked to poor survival in early N-stage SCCHN. The significance of these studies lies in the potential application of rap1GAP/MMP9 as biomarkers for selection of aggressive treatment in patients with early stage cancer. Furthermore, we identified a potential novel treatment target, EZH2, a master gene that silences rap1GAP and other tumor suppressors to promote growth and invasion. We are currently performing pre-clinical studies with an EZH2-inhibitor. Our results likely have broader implications, given that rap1GAP is a tumor suppressor in pancreatic cancer, thyroid cancer, and melanoma.

We optimized a mouse model of *human* SCCHN and used it for pre-clinical studies to evaluate the efficacy of an anti-tumor agent in vivo. This important mouse model is used by several groups to investigate SCCHN.

A recently completed study identified an “immunomic signature” in serum from SCCHN patients that is potentially applicable for early detection. We have also focused on saliva-based biomarkers for response to treatment and tumor recurrence. Monitoring SCCHN patients requires biopsies and expensive imaging. Due to new radiation techniques that protect the salivary glands, salivary function is recovered. Our novel findings showing that recovered saliva contains detectable proteins, opens up the possible use of saliva to monitor tumor recurrence. This study was highlighted on the journal cover and won an award. We are focusing on identifying a “signature” of differentially expressed proteins to monitor recurrence.

As a practicing clinician, translational scientist, and educator, I have made a significant impact in the field of oral pathology.

Rap1 and its Regulatory Proteins: The tumor suppressor, oncogene, tumor suppressor gene axis in head and neck cancer.

Nisha J. D'Silva [1,2,3]

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Footnotes

Financial support: This work was supported by NIDCR DE018512; DE019513; DE017977 grants (NJD).

Introduction

Squamous cell carcinoma of the head and neck (SCCHN) is the 6th most common cancer globally, with approximately 600,000 new cases a year (Leemans et al., 2011). At ~50%, the 5-year survival rate is poorer than breast cancer or melanoma. The poor disease-specific survival of SCCHN is due to late detection, which is a consequence of inadequate screening protocols (Bsoul et al., 2005).

Treatment for SCCHN is selected according to tumor stage, late stage lesions receive more aggressive treatment than early stage lesions which typically have a better prognosis (Brakenhoff, 2011). Despite receiving the standard of care, a significant number of early stage lesions progress to aggressive tumors. Identification of biomarkers that predict the biologic behavior and response to treatment of a lesion are required to facilitate selection of the most effective treatment and to serve as treatment targets.

Patients with late stage SCCHN are treated with aggressive surgery/radiation or chemotherapy/radiation (Brakenhoff, 2011). The surviving patient must confront challenges that include disfigurement, feeding and speech difficulties and poor quality of life. Due to minimal improvement in patient survival in four decades, the emphasis moved to dose escalation of radiation and chemotherapy. However, toxic effects limit the extent to which therapy can be intensified. The chemotherapy/radiation protocol led to organ preservation and improved quality of life. However, ~20-25% of patients do not respond to chemotherapy and are subsequently treated with surgery (Bauer et al., 2007; Urba et al., 2006). The delay in effective treatment and aggressive residual cells lead to poor patient survival (Bauer et al., 2007). Hence, new treatments must be identified to improve survival.

The areas of research in our laboratory are mechanistic, functional and translational studies on: a) serum and saliva biomarkers of early detection; b) tumor development and biomarkers of progression, i.e. which early stage lesions progress aggressively; c) biomarkers of chemoresistance. I will present our novel work on the significant role of rap1 and its regulatory proteins in SCCHN development and as biomarkers of progression.

Rap1 in SCCHN progression

Rap1 is a member of the ras subfamily of small GTPases that are critical players in the signaling pathways that control growth and differentiation. Ras is one of the most frequently mutated oncogenes in cancer but ras mutations in SCCHN are rare (Blumenschein, 2008; Suva et al., 2009). The sequence identity in the effector domains of ras and rap1 suggested either antagonistic or complementary activity in the same signaling cascade (Altschuler et al., 1995). This and the high expression of rap1 in keratinocytes (D'Silva et al., 2003), led to our focus on this protein in SCCHN.

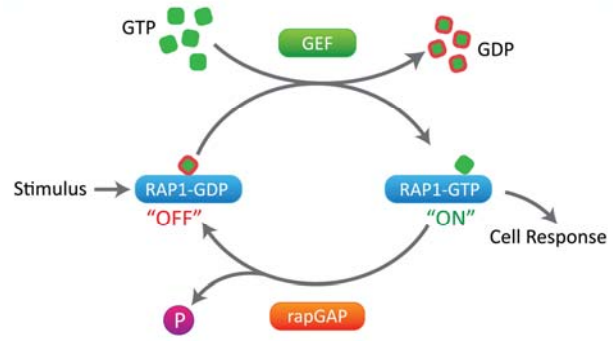

Figure 1: **Regulation of rap1.** Rap1 is activated by a stimulus such as galanin. The GDP to GTP exchange is facilitated by exchange factors, GEFs. Active rap1 triggers cell responses before being inactivated by rap1GAP.

Rap1 shuttles between inactive GDP- and active GTP-bound forms (Fig. 1). Activation of rap1 (rap1-GTP) is mediated by guanine nucleotide exchange factors (GEFs) including C3G, PDZ-GEF, Epac and CalDAG (Cironi et al., 2009). Inactivation of rap1 is mediated by GTPase activating proteins (rap1GAPs), which induce the endogenous GTPase activity of rap1. Given the critical role of proliferation and invasion in tumor development and progression, I will discuss i) the role of rap1 and rap1GAP in tumor progression; ii) regulation of rap1/ rap1GAP; iii) downstream targets of rap1/ rap1GAP; and iv) rap1/ rap1GAP-mediated biomarkers in SCCHN.

Active, GTP-bound rap1 is upregulated in SCCHN. Although rap1 is a ubiquitously expressed protein, till recently its function in epithelial cells, from which more than 80% of cancers are derived, was relatively unexplored and its function in the oral mucosa was unknown. In early studies, we showed that active rap1 regulates proliferation in normal keratinocytes and inactivation of rap1 reverses this phenotype (D'Silva et al., 2003). In malignant keratinocytes, which exhibit uncontrolled proliferation, active rap1 is highly upregulated and shuttles to the nucleus (Mitra et al., 2003). Active rap1 is increased by enhanced stimulation or decreased rap1GAP activity (Fig. 1). We showed that upregulation of active rap1 in SCCHN is due to reduced expression of rap1GAP and concurrent induction of rap1-mediated signaling pathways (Banerjee et al., 2011a; Zhang et al., 2006b).

Rap1GAP is a tumor suppressor gene and promotes invasion. Additional studies in SCCHN supported a novel function for rap1GAP as a tumor suppressor gene (Zhang et al., 2006b). Overexpression of rap1GAP in SCCHN delayed progression through the cell cycle, thereby reducing cell proliferation in vitro and tumor growth in vivo (Zhang et al., 2006b). Rap1 promotes rapid progression through the G1/S phase of the cell cycle via ERK/ MAPK activity (Zhang et al., 2006b).

In SCCHN, a small but significant number of patients with early stage disease will ultimately die of disease, despite appropriate treatment. These patients would likely benefit from more aggressive initial therapy. However, aggressive surgery and radiation treatment are physically and emotionally debilitating, and are not appropriate for all SCCHN. Therefore, the identification of biomarkers that are prognostic of tumor progression of early stage lesions and elucidation of the mechanism of action of these proteins will facilitate appropriate treatment selection. The tumor suppressor role for rap1GAP suggested that upregulation of rap1GAP in SCCHN would be prognostic of slower growing lesions with a favorable prognosis. However, subsequent in vitro and in vivo studies showed that rap1GAP promotes invasion, which promotes tumor extension into the underlying structures and spread to distant sites (Mitra et al., 2008). These findings are suggestive of a small but aggressive tumor. In fact, in SCCHN, rap1GAP promotes invasion by upregulating MMP2 and MMP9, metalloproteinases that facilitate degradation of the extracellular matrix (Mitra et al., 2008). Rap1GAP and MMP9 expression were correlated in human SCCHN, and high MMP9 correlated with poor disease specific survival (Mitra et al., 2008).

The outcome of our series of exciting studies on rap1/rap1GAP was that we identified a novel and critical tumor suppressor gene, rap1GAP, in SCCHN. Additionally, our findings suggested that pre-treatment screening for MMP9 in larger clinical trials may identify those patients with early N-stage lesions likely to benefit from aggressive treatment.

Rap1GAP is silenced by EZH2. The rap1GAP studies conclusively showed that therapeutic upregulation of rap1GAP in SCCHN would not be judicious due to the possibility of inhibiting tumor growth but promoting aggressive tumors by promoting invasion. Therefore, we investigated upstream mechanisms of regulation that concurrently silence rap1GAP and other tumor suppressor genes to promote multiple phenotypes such as invasion and proliferation. In SCCHN, methylation is an important epigenetic mechanism for silencing tumor suppressor genes (Banerjee et al., 2011b). Two critical mechanisms of silencing are EZH2 (Enhancer of zeste homolog 2)-mediated methylation of lysine residues in histone 3 and DNMT (DNA methyltransferase)-mediated methylation of CpG islands in the promoter region of genes. *EZH2,* a member of the polycomb repressive complex 2 (PRC2), *is a master regulatory gene* that silences multiple genes.

We showed that EZH2 is highly upregulated in SCCHN. (Banerjee et al., 2011b). In gene arrays, we identified rap1GAP as an EZH2 target. EZH2 silences rap1GAP via trimethylation of histone 3 at lysine 27. We also showed that EZH2 is required for DNMT methylation of the CpG islands in the promoter region of rap1GAP (summarized in Fig. 2). Since histone deacetylation is required for methylation, histone and promoter

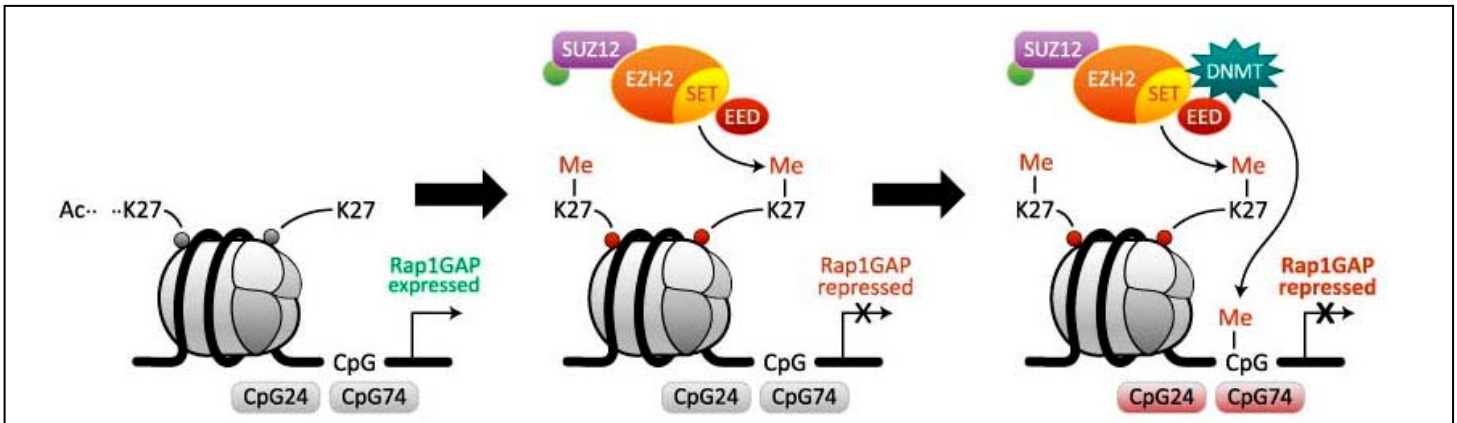

Figure 2: Methylation silences *rap1GAP*. Rap1GAP is expressed in the absence of methylation (left panel). Histone deacetylation facilitates polycomb complex (EZH2, SUZ12 and EED)-mediated histone methylation. The catalytically active SET domain of EZH2, methylates H3K27 resulting in chromatin compaction. Consequently, *rap1GAP* is silenced (middle panel). CpG islands in the promoter region of *rap1GAP* are methylated by DNA methyl transferase (DNMT), recruited by the PRC2 complex. This leads to profound silencing of *rap1GAP* (right panel). Methylation and silencing are denoted by red. Grey cylinder = histones, wrapping black line = DNA. Summary of results from (Banerjee et al., 2011b).

methylation of rap1GAP were inhibited by suberoylanilide hydroxamic acid (SAHA), a histone deacetylase inhibitor. Promoter methylation, mediated by DNMT, was blocked in the presence of 5-aza-2'-deoxycytidine (AZA), a DNMT inhibitor. Together, SAHA and AZA upregulated rap1GAP protein expression to an extent greater than either inhibitor alone (Banerjee et al., 2011b). Knockdown of EZH2 also reduced methylation of rap1GAP. Thus, trimethylation of Histone3 at lysine 27 and promoter hypermethylation suppress rap1GAP in SCCHN. We are currently validating other genes silenced by EZH2.

EZH2 promotes invasion, proliferation and tumor growth. In functional assays, EZH2 promoted proliferation, invasion and survival of immortalized, non malignant keratinocytes (Banerjee et al., 2011b). Furthermore, overexpression of EZH2 in non malignant keratinocytes suppressed rap1GAP expression and induced rap1 activation. Downregulation of EZH2 in malignant keratinocytes (SCCHN) had the reverse effect, i.e. inhibition of proliferation (Fig. 3A). Knockdown of EZH2 and rap1GAP "rescued" the proliferative phenotype of EZH2, establishing that EZH2 promotes proliferation by suppressing rap1GAP. These findings are important because EZH2 silences several tumor suppressor genes, but suppression of rap1GAP is essential for EZH2-mediated proliferation (Banerjee et al., 2011b). This emphasizes the critical role of rap1GAP in growth suppression in SCCHN.

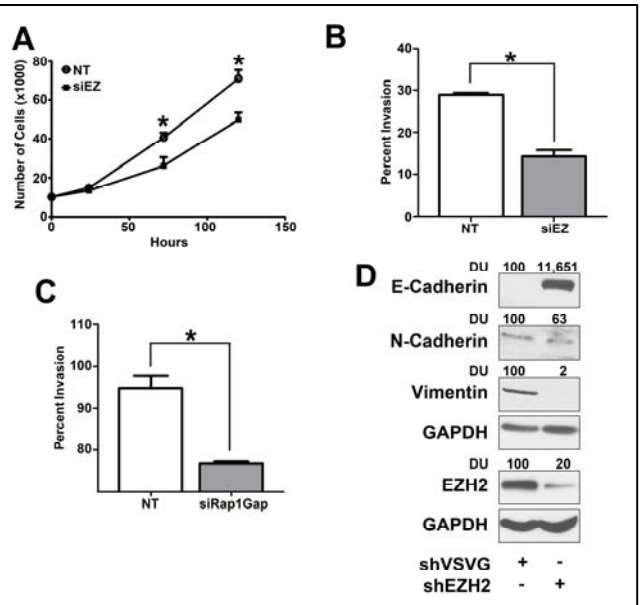

Figure 3: EZH2 promotes proliferation and invasion. UM-SCC-29 cells were transfected with non target siRNA or siEZH2 and proliferation (**A**) and invasion (**B**) assays were performed. **C**) UM-SCC-29 cells stably transduced with shEZH2 were subsequently transfected with non target siRNA or siRap1GAP. Invasion assays were performed (*p<0.05). **D**) Whole ell lysates from UM-SCC-29 cells stably transduced with shEZH2 were immunoblotted with E-Cadherin, N-Cadherin, Vimentin and EZH2 antibodies. GAPDH was used as a normalization control. Signal intensity was quantified by densitometric analysis, normalized to GAPDH and then expressed as percent of control.

We previously conclusively showed that rap1GAP in SCCHN promotes invasion (Mitra et al., 2008). Although EZH2 suppresses rap1GAP, EZH2 concurrently promotes invasion (Fig. 3B), suggesting that its effects on invasion are independent of rap1GAP. To investigate this possibility, invasion assays were performed in two independent SCCHN cell lines stably transduced with shEZH2 and transiently transfected with siRap1GAP. Knockdown of rap1GAP did not "rescue" EZH2-mediated invasion and instead, inhibited invasion (Fig. 3C). This is consistent with a role for rap1GAP in promoting invasion, as we reported previously (Mitra et al., 2008) and establishes that EZH2 promotes invasion via a rap1GAP-independent mechanism in SCCHN. Downregulation of EZH2, i.e. less invasion, decreased the mesenchymal markers, vimentin and N-cadherin, and increased E-cadherin (Fig. 3D). Together these studies support that EZH2 promotes tumor growth by silencing rap1GAP and promotes invasion via a rap1GAP-independent mechanism.

miR-101, which inhibits EZH2 is downregulated in SCCHN with consequent overexpression of EZH2, downregulation of rap1GAP and activation of rap1 (Banerjee et al., 2011b). This microRNA-mediated activation of rap1, via EZH2-mediated silencing of rap1GAP, is a novel mechanism of rap1 regulation. Thus a tumor suppressor-oncogene-tumor suppressor gene axis promotes the oncogenic phenotype in SCCHN via chromatin remodeling.

Rap1 may promote or inhibit invasion. Rap1, the target of rap1GAP, has two isoforms. Rap1A and Rap1B share 95% sequence identity, and are encoded by two different genes on chromosomes 1 and 12, respectively (Rousseau-Merck et al., 1990; Takai et al., 1993). The two Rap1 isoforms are expressed in the same cell (Mitra et al., 2003; Wittchen and Hartnett, 2011) where their functions may differ. A recent study in endothelial cells showed that Rap1A, but not Rap1B, promotes cell junction formation (Wittchen and Hartnett, 2011). In SCCHN, we showed that Rap1A promotes invasion via beta-catenin-induced transcriptional targets (Goto et al., 2010). Since Rap1GAP, which inhibits both Rap1 isoforms promotes invasion in SCCHN and rap1A

promotes invasion, it is likely that Rap1B, the dominant isoform, inhibits invasion. Our unpublished studies support this conclusion. Taken together our findings support a dual role for rap1 in invasion which may be related to the concurrent expression of other significant regulatory proteins, such as free β-catenin. The role of the microRNA-oncogene-tumor suppressor gene (mir101-EZH2-rap1GAP) axis and the dual role of rap1 are summarized in Fig. 4.

Galanin secreted by SCCHN promotes growth via rap1. Active rap1 is upregulated in SCCHN due to EZH2-mediated silencing of rap1GAP (Figs. 2, 3). Active rap1 is also increased by stimulation of galanin receptor 2, a G-protein coupled receptor (Banerjee et al., 2011a). In these novel mechanistic and functional studies, we conclusively showed that GalR2 promotes tumor progression via rap1-mediated Akt- and ERK-induced survival proliferation. These studies were significant because they elucidated a critical signaling pathway induced by galanin, which is secreted by SCCHN to create a positive feedback loop (Henson et al., 2005).

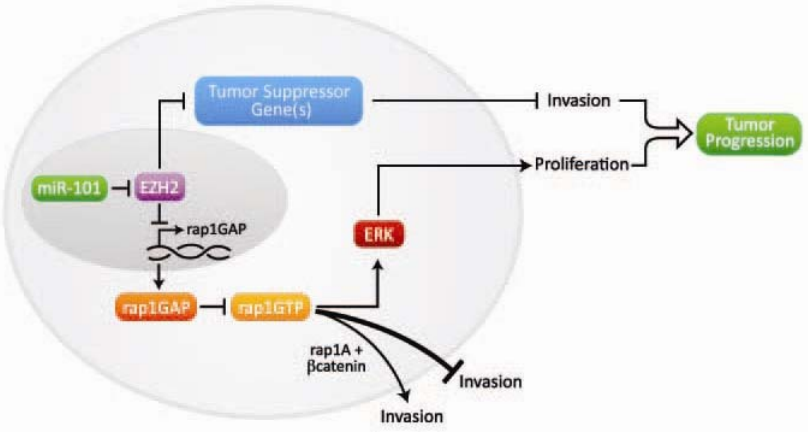

Figure 4: **Proposed mechanism**. EZH2 promotes tumor progression by silencing tumor suppressor genes. EZH2 promotes proliferation by silencing rap1GAP and promotes invasion via a rap1GAP-independent mechanism. miR-101 is reduced in SCCHN thereby leading to overexpression of EZH2. Rap1GAP promotes invasion but the individual rap1 isoforms or the concurrent expression of critical signaling molecules, likely modulate this effect.

Conclusion

Targeted therapy against single growth factors and their receptors had limited success in SCCHN. This is likely due to simultaneous alteration of multiple genes that affect tumor growth, spread and treatment resistance, or cross-talk between signaling pathways. Our findings emphasize the need for carefully defining the signaling pathways regulated by a putative treatment target, and correlating functional effects with clinical outcomes prior to developing inhibitors. Though rap1GAP inhibits tumor growth, it also promotes invasion in SCCHN. Therefore, therapeutic targeting of rap1GAP in tumors would not be judicious due to the possibility of fostering small but aggressive tumors. However, our studies suggest that methylation is an attractive treatment target because it silences multiple tumor suppressor genes via chromatin remodeling. Furthermore, our studies helped us identify potential biomarkers for tumor progression and suggested that pre-treatment screening for MMP9 in larger clinical trials may identify those patients with early N-stage lesions likely to benefit from aggressive treatment.

Although the studies presented here are presented in the context of SCCHN, our results may have borader implications, given that rap1GAP is a tumor suppressor in pancreatic cancer, thyroid cancer, and melanoma (Tsygankova et al., 2007; Zhang et al., 2006a; Zheng et al., 2009).

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