37TH ANNUAL RESEARCH DAY

ABSTRACTS

Friday, April 1, 2022

Post Docs
Title: PKCδ controls the DNA damage response to irradiation through regulation of chromatin accessibility and DNA double stranded break repair
Trisiani Affandi, Angela M. Ohm, Ami Haas, Joshua C. Black, Gregory M. Wright, Jordan T. Speidel and Mary E. Reyland

Purpose: Patients treated with irradiation (IR) for head and neck cancer (HNC) often sustain collateral damage to non-tumor tissues in the oral cavity. Protein kinase C delta (PKCδ) regulates IR-induced apoptosis in salivary acinar cells, and inhibition of PKCδ preserves salivary gland function in mouse models of head and neck irradiation without protecting the tumor. We hypothesize that inhibition of PKCδ suppresses apoptosis by increasing double stranded break (DSB) repair.

Methods: Formation of micronuclei was quantified to investigate genomic instability. DNA damage was analyzed by γH2AX foci quantification and Comet assay. In vivo fluorescent reporter assay was used to directly quantify non-homologous end joining (NHEJ) and homologous recombination (HR). Chromatin remodeling was investigated using a micrococcal nuclease (MNase) assay and by analysis of histone modifications using mass spectrometry.

Results: RPE cells that stably overexpress PKCδ show increased chromosomal instability, indicating that PKCδ has a negative impact on genome integrity. We show that endogenous PKCδ regulates chromatin accessibility and suppresses DSB repair. Contrarily, DNA repair is increased in PKCδ-depleted cells as evidenced by more rapid resolution of IR-induced γH2AX foci and a more rapid decrease in DNA damage. Depletion of PKCδ increases DNA repair through both NHEJ and HR pathways. Depletion of PKCδ is associated with increased MNase sensitivity, suggesting a more open chromatin, while overexpression of PKCδ decreases MNase sensitivity. In PKCδ-depleted cells, H3K23ac is decreased and H3K36me2 is increased, both histone marks are associated with DNA repair.

Conclusions: Our data suggests a novel mechanism for control of apoptosis by PKCδ mediated through regulation of chromatin accessibility and DNA repair. Understanding the mechanism by which PKCδ regulates DNA damage-induced apoptosis may allow us to identify new targets for radioprotection of oral tissues in HNC patients.

Funding Source: NIH/NIDCR R01DE015648 and R01DE027517 to MER
Title: Characterization of PDGFRα/β heterodimer-specific dynamics
Maria B. Campana, Madison A. Rodgers, and Katherine A. Fantauzzo

Purpose: Signaling through the platelet-derived growth factor receptors (PDGFRs) plays a critical role in human craniofacial development. The PDGFR family consists of two receptors, PDGFRα and PDGFRβ, that dimerize to form PDGFRα homodimers, PDGFRα/β heterodimers, and PDGFRβ homodimers. We have shown that PDGFRα and PDGFRβ genetically and physically interact in the craniofacial mesenchyme to form functional heterodimers with unique signal molecule binding properties from homodimeric receptor complexes. It has previously been impossible to study dimer-specific dynamics for PDGFRs because commonly-used antibody-based approaches do not allow for the visualization and purification of individual PDGFR dimers. Here, we highlight ongoing experiments that overcome these previous limitations in studying PDGFRα/β heterodimers using bimolecular fluorescence complementation (BiFC).

Methods: We generated a cell line stably expressing C-terminal fusions of PDGFRα and PDGFRβ with BiFC fragments corresponding to the N-terminal (V1) and C-terminal (V2) regions of the Venus fluorescent protein, respectively.

Results: We confirmed heterodimerization of the receptors and bimolecular fluorescence complementation upon PDGF-BB ligand stimulation of these cells via fluorescence microscopy and immunoprecipitation with a nanobody (GFP-Trap) that recognizes an epitope spanning the V1/V2 interface. We found that these receptors heterodimerize quickly in response to PDGF-BB ligand, with increased levels of receptor autophosphorylation at early time points of ligand treatment. We further discuss current studies to examine the intracellular trafficking dynamics of PDGFRα/β heterodimers following activation, identify PDGFR dimer-specific interacting proteins using mass spectrometry-based proteomics, and examine the spatiotemporal activation of PDGFRα/β heterodimers during craniofacial development in vivo using a novel mouse model.

Conclusions: These studies will impart valuable insight into the molecular mechanisms by which biological specificity is introduced downstream of PDGFR activation to regulate craniofacial development.
Title: Auriculocondylar Syndrome 2 results from dominant negative action of PLCB4 variants
Stanley M. Kanai, Caleb Heffner, Timothy C. Cox, Michael L. Cunningham, Francisco A. Perez, Aaron Bauer, Philip Reigan, Cristan Carter, Stephen A. Murray and David E. Clouthier

Purpose: Auriculocondylar Syndrome 2 (ARCND2) is a rare autosomal dominant craniofacial malformation syndrome linked to multiple genetic variants in the coding sequence of phospholipase C β4 (PLCB4). PLCB4 is a direct signaling effector of the Endothelin Receptor Type A (EDNRA)-Gq/11 pathway, which establishes the identity of neural crest cells (NCCs) that form lower jaw and middle ear structures. However, the functional consequences of PLCB4 variants on EDNRA signaling is not known.

Methods: Here, we used multiple signaling reporter assays, including a novel Bioluminescence Resonance Energy Transfer (BRET) assay, to test the functional consequences of PLCB4 variants. We also modeled one PLCB4 variant in F0 mouse embryos using CRISPR/Cas9 to determine whether it will recapitulate ARCND2 phenotypes. Finally, we performed comparative anatomy to determine whether craniofacial changes observed in our ARCND2 mouse model is also present in human individuals with ARCND2.

Results: Our signaling reporter assays demonstrated that PLCB4 variants resulting from missense mutations exert dominant negative interference on EDNRA signaling. In addition, ARCND2 mouse models have facial defects recapitulating those observed in hypomorphic Ednra mouse models, including a bone that we identify as an atavistic change in the posterior palate/oral cavity. Remarkably, we have identified a similar osseous phenotype in a child with ARCND2.

Conclusions: Our results identify the disease mechanism of ARCND2, demonstrate that the PLCB4 variants cause craniofacial differences and illustrate how minor changes in signaling within NCCs may have driven evolutionary changes in jaw structure and function.

Funding: This work was supported in part by the National Institutes of Health (DE025862-03S1 to D.E.C).
Title: Prdm3 and Prdm16 genetically interact in the mammalian neural crest during craniofacial development
Lomeli C. Shull, Kristin B. Artinger

Purpose: The gene regulatory networks and signaling pathways controlling craniofacial development must be tightly controlled, as alterations can contribute to the etiology of congenital birth defects. We previously showed that two PRDM histone methyltransferases, Prdm3 and Prdm16, facilitate cranial neural crest chondrocyte differentiation and maturation during zebrafish craniofacial development by balancing temporal and spatial Wnt/β-catenin transcriptional activity. Here, we sought to determine whether the mechanisms of craniofacial chondrocyte development observed in zebrafish are conserved in mammals.

Methods: Prdm3 and Prdm16 were conditionally deleted in the murine neural crest lineage using the Wnt1-Cre driver (Prdm3/fl/fl;Wnt1-Cre+/Tg and Prdm16/fl/fl;Wnt1-Cre+/Tg). To assess whether Prdm3 and Prdm16 genetically interact, double floxed Prdm3/fl/fl;Prdm16/fl/fl mice and bred them to Prdm3/fl/+;Prdm16/fl/+;Wnt1-Cre+/Tg mice to create all allelic combinatorial mutants. Craniofacial phenotypes were assessed by alcian blue and alizarin red staining at embryonic day (E) 18.5. Histology was performed at E15.5 on coronal sections through the palate.

Results: Homozygous loss of Prdm3 results in subtle craniofacial phenotypes, namely mild anterior mandibular hypoplasia. Prdm16/fl/fl;Wnt1-Cre+/Tg presented with a variety of craniofacial abnormalities, including snout extension defects, anterior mandibular hypoplasia, and severe hypoplasia of the middle ear structures. A subset of these animals (20%) develop a secondary cleft palate. Mandibular hypoplasia observed in both mutants likely results from abnormal chondrocyte maturation in the developing Meckel’s cartilage that supports mandible formation. Prdm3/fl/fl;Prdm16/fl/+;Wnt1-Cre+/Tg mutants develop a penetrable secondary cleft palate, while double Prdm3/fl/fl;Prdm16/fl/fl;Wnt1-Cre+/Tg mutants present with a high arched palate, unorganized palatal rugae, abnormal maxillary bone structures, and a severely hypoplastic mandible indicative of altered mineralization in these tissues.

Conclusions: Prdm3 and Prdm16 have important roles in the mammalian neural crest during craniofacial development. Future studies include dissecting how these factors control the formation of the mandible and subsequently how this might influence the development of the palate.

Funding source: NIH/NIDCR F32 DE029099 and R01 DE024034