Hcfc1 AND Ogt MEDIATE ZEBRAFISH CNS REGENERATION THROUGH HIPPO/Yap SIGNALING

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by

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CERTIFICATE

This is to certify that the thesis titled "**Hcfc1 and Ogt regulate CNS regeneration through Hippo/Yap signaling**" submitted by **Ms. Priyanka Prakash Srivastava** to the Indian Institute of Technology Delhi for the award of the degree of "Doctor of Philosophy" is a record of the Bonafide research carried out by her, which has been prepared under my supervision and guidance in conformity with the rules and regulations of the Indian Institute of Technology Delhi, India. The results prescribed in it have not been submitted in part or full to any other University for the award of any degree or diploma.

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Priyanka Prakash Srivastava

ABSTRACT

Tissue regeneration in the central nervous system is an intricate and complex process that requires tight molecular regulation. However, the precise transcriptional co-regulators involved in this process remain poorly understood. In this study, using the zebrafish model, we identify Host Cell Factor-1 (Hcfc1) and O-linked N-acetylglucosamine transferase (Ogt) as crucial regulators of regenerative processes in both the brain and retina. Host Cell Factor-1, encoded by the *hcfc1* gene in mouse and zebrafish (*HCFC1* in human). The protein is referred to as HCF-1 in mouse and HCFC1 in human. This conserved transcriptional co-regulator is essential for transcriptional regulation and cell cycle progression. It undergoes extensive O-GlcNAcylation and site-specific proteolytic cleavage by Ogt, which has dual glycosyltransferase and protease activities. Our work shows that Hcfc1 is essential for both development and regeneration. Knockout and knockdown of Hcfc1 caused various developmental defects and were lethal. Further, our experiments show that knockdown of zebrafish Hcfc1 paralogs (Hcfc1a, Hcfc1b, and both together) in the retina, along with pharmacological inhibition of Ogt using OSMI-1 in both brain and retinal tissues, leads to a significant impairment in regenerative capacity. Further analysis revealed that loss of Hcfc1 and Ogt activity shows a marked reduction in Yap levels, suggesting their vital role in maintaining regenerative signaling. Interestingly, we found that overexpression of a constitutively active *yap5SA* construct in the retina could rescue proliferation defects caused by the loss of Hcfc1 and Ogt activity, reinforcing the connection between these factors and regenerative outcomes. These findings provide new insights into the molecular mechanisms underlying neural regeneration and suggest that modulation of Hcfc1 and Ogt activity could be a valuable approach for promoting regenerative processes in zebrafish and other vertebrates.

सारांश

केंद्रीय तंत्रिका तंत्र में ऊतक पुनर्जनन एक जटिल प्रक्रिया है जिसके लिए कड़े आणविक नियमन की आवश्यकता होती है। हालाँकि, इस प्रक्रिया में शामिल सटीक ट्रांसक्रिप्शनल सह-नियामक अभी भी पूरी तरह से समझ में नहीं आए हैं। इस अध्ययन में, ज़ेब्राफिश मॉडल का उपयोग करते हुए, हमने होस्ट सेल फैक्टर-1 (Hcfc1) और O-लिंक्ड N-एसिटाइलग्लुकोसामाइन ट्रांसफ़ेरेज़ (Ogt) को मस्तिष्क और रेटिना दोनों में पुनर्योजी प्रक्रियाओं के महत्वपूर्ण नियामकों के रूप में पहचाना है। होस्ट सेल फैक्टर-1, चूहे और ज़ेब्राफिश (मानव में HCFC1) में hcfc1 जीन द्वारा एन्कोड किया गया है। इस प्रोटीन को चूहे में HCF-1 और मानव में HCFC1 कहा जाता है। यह संरक्षित ट्रांसक्रिप्शनल सह-नियामक ट्रांसक्रिप्शनल नियमन और कोशिका चक्र प्रगति के लिए आवश्यक है। यह Ogt द्वारा व्यापक O-GlcNAcylation और साइट-विशिष्ट प्रोटियोलिसिस से गुजरता है, जिसमें दोहरी ग्लाइकोसिलट्रांसफेरेज़ और प्रोटीएज़ गतिविधियाँ होती हैं। हमारा कार्य दर्शाता है कि Hcfc1 विकास और पुनर्जनन दोनों के लिए आवश्यक है। Hcfc1 के नॉकआउट और नॉकडाउन से विभिन्न विकासात्मक दोष उत्पन्न हुए और ये घातक थे। इसके अलावा, हमारे प्रयोगों से पता चलता है कि ज़ेब्राफ़िश Hcfc1 पैरालॉग्स (Hcfc1a, Hcfc1b, और दोनों एक साथ) को रेटिना में नॉकडाउन करने के साथ-साथ मस्तिष्क और रेटिना के ऊतकों में OSMI-1 का उपयोग करके Ogt के औषधीय निषेध से पुनर्योजी क्षमता में उल्लेखनीय कमी आती है। आगे के विश्लेषण से पता चला कि Hcfc1 और OGT गतिविधि की हानि से Yap के स्तर में उल्लेखनीय कमी देखी गई, जो पुनर्योजी संकेतन को बनाए रखने में उनकी महत्वपूर्ण भूमिका का संकेत देता है। दिलचस्प बात यह है कि हमने पाया कि रेटिना में एक संवैधानिक रूप से सक्रिय yap5SA संरचना की अधिक अभिव्यक्ति, Hcfc1 और Ogt गतिविधि की हानि के कारण होने वाले प्रसार दोषों को बचा सकती है, जिससे इन कारकों और पुनर्योजी परिणामों के बीच संबंध मजबूत होता है। ये निष्कर्ष तंत्रिका पुनर्जनन के अंतर्निहित आणविक तंत्रों में नई अंतर्दृष्टि प्रदान करते हैं और सुझाव देते हैं कि एचसीएफसी1 और ओजीटी गतिविधि का मॉड्यूलेशन ज़ेब्राफिश और अन्य कशेरुकियों में पुनर्योजी प्रक्रियाओं को बढ़ावा देने के लिए एक मूल्यवान दृष्टिकोण हो सकता है।

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ABBREVIATIONS

Blbp	Brain lipid binding protein
BMP	Bone morphogenetic protein
BrdU	5-bromo-2'-deoxyuridine
CNS	Central nervous system
Dkk1b	Dickkopf WNT signaling pathway inhibitor 1b
GFAP	Glial fibrillary acidic protein
HSV	Herpes simplex virus
Id1	Inhibitor of DNA binding
NCC	Neural crest cell
NPC	Neural precursor cell
NSC	Neural stem cell
RGC	Radial glial cell
Shh	Sonic hedgehog
VZ	Ventricular zone
Wnt	Wingless related integration site
OGT	O-Linked N-Acetylglucosamine (GlcNAc) Transferase
ID	Intellectual disability
KO	Knockout
KD	Knockdown
MMACHC	Metabolism of cobalamin associated C
Asxl1	Additional sex comb like transcriptional regulator1
THAP11	Thanatos-associated protein domain containing 11
EGFR	Epidermal growth factor receptor
HCF-1/HCFC1	Host cell factor-1
YAP	Yes associated protein
HBM	HCF-1 binding motif

Sox2	Sex determining region box-2
NeuN	Neuronal nuclear protein
MS222	Tricaine methanesulfonate
HE	Hematoxylin-Eosin
PCNA	Proliferating cell nuclear antigen
Dpl	Days post lesion
Dpi	Days post injury
Dpf	Days post fertilization
Hpf	Hours post fertilization
kDa	Kilodalton
DAPI	4',6-diamidino-2-phenylindole
Tg	Transgenic
NeuroD1	Neurogenic differentiation 1
PTM	Post translational modification
TPR	Tetratricopeptide repeat
RTT	Rett syndrome
DS	Downs Syndrome
MG	Muller Glia
MGPC	Muller glia-derived progenitor cells
TMG	Thiamet G
PBMC	Peripheral blood mononucleocytes
CC	Cellular component
MF	Molecular function
TBI	Traumatic brain injury
Olig2	Oligodendrocyte transcription factor 2
Yap	Yes-associated protein
PCNA	Proliferating cell nuclear antigen