CHAPTER 19

GENETICS

Doctoral Theses

01. GANDHI (Sanchit)
Molecular Characterization of the Hypoxic Tumour Microenvironment in Glioma and Gastric Cancer.
Supervisor: Prof. Tapasya Srivastava
Th 27487

Abstract

Cellular and molecular heterogeneity are key drivers of tumour progression responding and contributing to dynamic changes in the tumour microenvironment. Hypoxia is a key feature of the tumour microenvironment. Hypoxic tumour microenvironment, captained by transcription factors of HIF family, is associated with tumour-favouring processes that include cell survival, angiogenesis, invasion, metastasis and many more. We attempted to study the hypoxic-mediated biochemical, molecular and cellular mechanisms in solid tumours. Gliomas are associated with high mortality and morbidity. Long non-coding RNAs landscape in the development and progression into malignant stages has recently captured the attention of researchers. Insights into the functions of lncRNAs will help identify novel diagnostic/prognostic markers. In our study, identification of hypoxia-relevant lncRNAs using in-house tool "GenOx" was coupled with molecular, functional and interactome-based analyses followed by validation in A172 cell line. SNHG1 and TP53TG1 emerged as novel prognostic biomarkers, highly relevant in glioma progression. SNHG1 expression in tumour is distinct from glioma stem cells and in hypoxia, positioning the downregulation of SNHG1 to be associated with worsened prognosis. Gastric cancer is the fifth most malignant cancer. Its molecular etiology and new discoveries are accelerating treatment strategies to overcome poor prognosis. We endeavoured to characterize the functional role of SERTAD1 in the hypoxic tumour microenvironment. An exploration of the available datasets of tumour versus normal showed high expression of SERTAD1 in gastric cancer was associated with poor prognosis, similar to our observation in hypoxia versus normoxia. Transient knockdown of SERTAD1 led to reduction in proliferation and dysregulation of key hallmark genes, pointing to the potential oncogenic role SERTAD1 plays in the tumour microenvironment. We are currently focusing on discovering peptide inhibitors of SERTAD1. Studying the tumour microenvironment will provide clues for targeting novel molecules that will definitely overcome the current therapeutic and diagnostic/prognostic challenges in glioma and gastric cancer.

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02. PRIYANKA

Plant Regeneration, *Agrobacterium*-Mediated Transformation and Over-Expression of *miR397b* for Yield Improvement in Black Rice (*Oryza sativa*, cv. Chakhao Amubi).

Supervisors: Prof. P.K. Burma and Prof. M.V. Rajam

Th 27886

Abstract

The Chakhao cultivars are important rice varieties of North-eastern India called black rice due to the presence of anthocyanin pigments. Despite high demand and nutritional value of black rice, its yield is very poor. Therefore, as a prelude to develop transgenic black rice with improved yield by transferring yield-related genes, initially an efficient protocol was optimized for plant regeneration using the embryogenic calli of black rice (cv. Chakhao amubi). Among various combinations, MS medium supplemented with 3 mg/L 2-4, D, 0.4% phytagel, 3% maltose showed highest callus induction frequency (96%). Further, an efficient and improved plant regeneration was achieved by using the combination of plant hormones, viz., benzyladenine purine (BAP), napthelene-acaetic-acid (NAA) and kinetin (Kn) at a concentration of 2.0 mg/L, 1 mg/L and 0.2 mg/L respectively, with 0.8% agarose. Black rice is recalcitrant for genetic transformation and regeneration of transformants due to its pigment. Since, there are a limited genetic transformation protocols available for black rice, and therefore crop improvement efforts have not been made so much progress. Here, we also standardized an efficient Agrobacterium-mediated transformation protocol with EHA105 strain, and transformation efficiency was found to be 54% with 33% of regeneration frequency of transformants. In addition, the established transformation and regeneration protocol was used to raise black rice primary (T0) transformants with OsmiR397b, and they were advanced to T1 and T2 generations, which were confirmed by molecular analyses. Phenotypic evaluation of the T1 transgenic lines showed differences in flowering time, panicle architecture and grain size as compared to the wild-type plants. Further, the increase in the number of primary and secondary branches led to the increased spikelet number with a positive correlation with grain yield. SEM analysis of stem revealed the increase in number of vascular bundles and pith diameter in transgenic lines as compared to the wild-type plants. Interestingly, OsmiR397b down-regulates its target laccase family genes and increase sensitivity to brassinosteroids in all the transgenic lines. These results demonstrated that OsmiR397b is efficient in influencing yield traits, and may also serve as an important target for the improvement of other cultivars of black rice and other crops.

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03. YADAV (Vipul)

Elucidating the Unction of Pina, a Parvulin PPiase, in Dictyostelium Discoideum using Gene Deletion and Mutant Studies.

Supervisor: Dr. Arona Naorem

Th 27489

Abstract

Many secreted factors play important role starting from sensing starvation to early and late developmental processes in Dictyostelium discoideum. We found that PinA,

a D. discoideum homolog of Ess1/Pin1 parvulin-type peptidyl prolyl cis/trans isomerase, is important for growth and development as cells lacking pinA (pinA-) exhibit growth and developmental phenotypes such as precocious aggregation with numerous small aggregates, abnormal cell patterning etc. This thesis describes the investigations done to understand the role of PinA in cellular processes of D. discoideum using pinA deletion (pinA-) and pinA mutants. Error prone PCR was used to generate pinA mutants having one or two amino acid changes altering protein structure and were confirmed by their inability to substitute either Ess1 or PinA in ess1H164R ts mutant or pinA- cells respectively. Further overexpression in wild-type Ax2 cells showed that these mutants exhibited excessive aggregation stream break-up which was not seen in pinA- cells. Development using conditioned medium and low cell density revealed that pinA cells oversecrete cAMP resulting in formation of numerous aggregation centres which was rescued by addition of exogenous adenosine and precocious aggregation. Cell movement studies found that PinA may regulate cell-cell and cell-substrate interactions to modulate normal cell movement during aggregation stream formation. Analyzing processes in late developmental stages also revealed that PinA is required for cell differentiation. Codevelopment studies found that diffusible/secreted factors from Ax2 cells are not sufficient to suppress phenotypes of pinA- or cells expressing pinA mutants. mRNA expression profiling and protein localization studies pointed that PinA may regulate early and late developmental stages by modulating the expression of genes. Taken together, this study finds PinA can regulate in D. discoideum growth and development by modulating secreted factors for sensing starvation and aggregation, cAMP metabolism, cell-movement, chemotaxis and other late developmental processes.

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