FEATURES

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Mechanisms Regulating Acquisition of Platelet–Derived Factor V/Va by Megakaryocytes 2121

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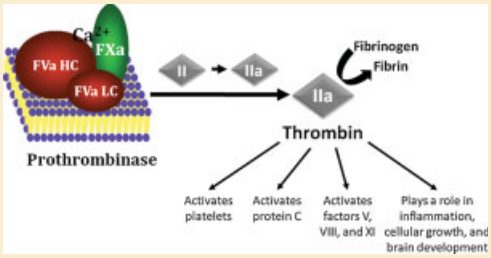
Factor Va serves as the nonenzymatic protein cofactor for the prothrombinase complex, which converts prothrombin to thrombin in the events leading to formation of a hemostatic plug. Several observations support the concept that platelet-derived factor V/Va is physically and functionally distinct and plays a more important role in thrombin generation at sites of vascular injury as compared to its plasma counterpart. Platelet-derived factor V/Va is generated following endocytosis of the plasma-derived molecule by the platelet precursor cells, megakaryocytes, via a two receptor system consisting of low density lipoprotein (LDL) receptor-related protein-1 (LRP-1) and an unidentified specific "binding site". More recently, it was suggested that a cell surface-expressed β-galactoside binding protein, galectin-8, was involved in factor V endocytosis. Endocytosed factor V is trafficked through the cell and retailored prior to its storage in α-granules. Given the essential role of platelet-derived factor Va in clot formation, understanding the cellular and molecular mechanisms that regulate how platelets acquire this molecule will be important for the treatment of excessive bleeding or clotting.



Role and Regulation of Myeloid Zinc Finger Protein 1 in Cancer 2146

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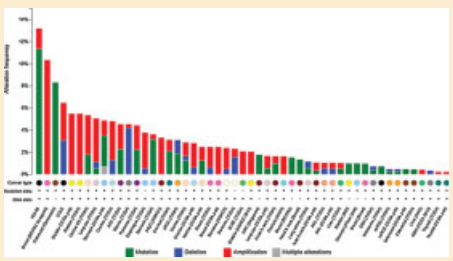
Myeloid zinc finger 1 (MZF1) belongs to the SCAN-Zinc Finger (SCAN-ZF) transcription factor family that has recently been implicated in a number of types of cancer. Although the initial studies concentrated on the role of MZF1 in myeloid differentiation and leukemia, the factor now appears to be involved in the etiology of major solid tumors such as lung, cervical, breast, and colorectal cancer. Here we discuss the regulation of MZF1 that mediated its recruitment and activation in cancer, concentrating on posttranslational modification by phosphorylation, and sumoylation, formation of promyelocytic leukemia nuclear bodies and its association with co-activators and co-repressors.



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Post-Translational Modifications of Histones in Human Sperm

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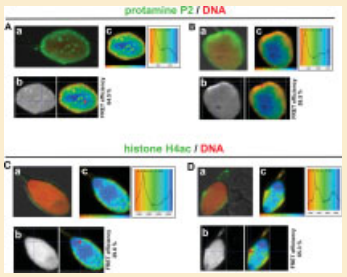
We examined the levels and distribution of post-translationally modified histones and protamines in human sperm. Using western blot immunoassay, immunofluorescence, mass spectrometry (MS), and FLIM-FRET approaches, we analyzed the status of histone modifications and the protamine P2. Among individual samples, we observed variability in the levels of H3K9me1, H3K9me2, H3K27me3, H3K36me3, and H3K79me1, but the level of acetylated (ac) histones H4 was relatively stable in the sperm head fractions, as demonstrated by western blot analysis. Sperm heads with lower levels of P2 exhibited lower levels of H3K9ac, H3K9me1, H3K27me3, H3K36me3, and H3K79me1. A very strong correlation was observed between the levels of P2 and H3K9me2. FLIM-FRET analysis additionally revealed that acetylated histones H4 are not only parts of sperm chromatin but also appear in a non-integrated form. Intriguingly, H4ac and H3K27me3 were detected in sperm tail fractions via western blot analysis. An appearance of specific histone H3 and H4 acetylation and H3 methylation in sperm tail fractions was also confirmed by both LC-MS/MS and MALDI-TOF MS analysis. Taken together, these data indicate that particular post-translational modifications of histones are uniquely distributed in human sperm, and this distribution varies among individuals and among the sperm of a single individual.



Human Milk MicroRNA and Total RNA Differ Depending on Milk Fractionation

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MicroRNA have been recently discovered in human milk signifying potentially important functions for both the lactating breast and the infant. Whilst human milk microRNA have started to be explored, little data exist on the evaluation of sample processing, and analysis to ensure that a full spectrum of microRNA can be obtained. Human milk comprises three main fractions: cells, skim milk, and lipids. Typically, the skim milk fraction has been measured in isolation despite evidence that the lipid fraction may contain more microRNA. This study aimed to standardize isolation ofmicroRNA and total RNA fromall three fractions of human milk to determine the most appropriate sampling and analysis procedure for future studies. Three different methods from eight commercially available kits were tested for their efficacy in extracting total RNA and microRNA from the lipid, skim, and cell fractions of human milk. Each fraction yielded different concentrations of RNA and microRNA, with the highest quantities found in the cell and lipid fractions, and the lowest in skim milk. The column-based phenol-free method was the most efficient extraction method for all three milk fractions. Two microRNAs were expressed and validated in the three milk fractions by qPCR using the three recommended extraction kits for each fraction. High expression levels were identified in the skim and lipid milk factions for these microRNAs. These results suggest that careful consideration of both the human milk sample preparation and extraction protocols should be made prior to embarking upon research in this area.