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MEETING REPORT

Report on the Workshop "New Technologies in Stem Cell Research," Society for Pediatric Research, San Francisco, California, April 29, 2006

INTRODUCTION: This is a meeting report on the workshop "New Technologies in Stem Cell Research," which was presented to pediatric residents, fellows, and faculty at the Society for Pediatric Research meeting in San Francisco, California, on April 29, 2006. Four speakers presented an overview of selected topics related to the current status of methods used to study stem cells. The topics presented at the workshop focused on RNA interference, mesenchymal stem cells, expression analysis, and gene therapy. In the first report, Drs. Jerry Cheng and Kathleen Sakamoto summarize the application of RNA interference in stem cells. Second, Dr. Edwin Horwitz describes basic approaches to the isolation and purification of mesenchymal stem cells. Third, Drs. Stanislav Karsten, Lorelei Shoemaker, and Harley I. Kornblum discuss methods in expression analysis of stem cells. Fourth, Dr. Punam Malik reports on the use of gene therapy for hemoglobinopathies using autologous stem cells.

Disclosure of potential conflicts of interest is found at the end of this article.

Key Words. Stem cells • RNA interference • Expression profiles • Neural stem cells • Mesenchymal stem cells • Purification Gene therapy • Hematopoietic stem cells

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RNA Interference and Stem Cells

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RNA interference (RNAi) is a powerful tool with which to study gene function, especially in stem cells. Small interfering RNAs (siRNAs) can effectively be introduced either with a vehicle or through viral vectors to transiently or stably inhibit the expression of a particular gene target. Much is known about the optimization of siRNAs and method of delivery in mammalian cells. In this review, we discuss design considerations for siRNAs, methods of delivery, optimization of siRNAs, applications to study genes in stem cells, therapeutic applications, and remaining hurdles. With recent advances in RNAi, it is likely that application of this technology will increase in the future.

Disclosure of potential conflicts of interest is found at the end of this article.

Key Words. RNA interference • Stem cells • Lentivirus

Fundamentals of MSC Isolation and Purification

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Methods in Expression Analysis of Stem Cells

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Recent advances in stem cell technology have opened the door to the study of stem cell biology, including mechanisms underlying the fundamental properties of stem cells: self-renewal and cell fate. These analyses can be greatly enhanced by large-scale studies of gene and protein expression. Such studies can be used to categorize stem cells and their progeny, as well as to determine specific genes, proteins, and molecular pathways involved in functional processes. This review provides examples of how expression analysis can be used by the stem cell biologist, as well as methodological guidance in determining what questions can be asked. Furthermore, we provide descriptions of currently available microarray platforms and analysis tools.

Disclosure of potential conflicts of interest is found at the end of this article.

Key Words. Genomics • Proteomics • Microarray • Large scale • Methods

Gene Therapy for Hemoglobinopathies Using Autologous Hematopoietic Stem Cells

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Key Words. Thalassemia • Hemoglobin • Retroviral vectors • Lentiviral vectors

Hemoglobin disorders constitute the most common single-gene disorders that are potentially amenable to gene therapy. However, retroviral vectors carrying the human β -globin cassette are notoriously unstable, express the transgene at low levels, or are unable to hold large erythroid regulatory elements. In the past 5 years, tremendous progress has been made in this field with the use of lentiviral vectors. Our laboratory investigated lentiviral vectors for erythroid lineage-specific expression, long-term expression, and silencing following transduction of hematopoietic stem cells. In addition, we have been able to overcome the chromatin position effects with insulated self-inactivating lentiviral vectors that have increased probability of expression from individual integrants and reduced clonal variegation in expression in long-term transplanted mice. We have shown complete correction of the human thalassemia phenotype in vitro and in xenografts in the red blood cell progeny of CD34⁺ cells from patients with β -thalassemia major. This article provides a concise review of the current status of gene therapy for hemoglobin disorders and the steps needed for safe human clinical trials.

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