Single-Cell Mutation Monitoring Systems

Methodologies and Applications

Edited by

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Research Triangle Park, North Carolina

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Preface

There is general agreement that increased environmental pollution poses a potential health hazard to humans and that effective control of such genetic injury requires monitoring the exposed individuals for genetic damage and identifying chemicals that may cause mutation or cancer. Tests available for identifying mutagens or carcinogens range from relatively simple, rapid assays in prokaryotes and test systems utilizing mammalian cells in tissue culture to highly elaborate tests in intact animals. No single test can provide data for an unequivocal assessment of the mutagenicity of a given chemical and the risk it might pose to human health. A tier approach, therefore, was suggested for mutagenicity testing in which the suspected agents would be initially evaluated with simple, inexpensive tests that would give qualitative results. Chemicals found to be positive in the first-tier testing would then be evaluated with more complex tests, including those based on mammalian cells in culture. Testing in the final tier requires whole-animal studies, and is expensive and time-consuming, and even the results from these studies need to be extrapolated for human risk assessment.

The mutation systems based on whole animals require scoring large numbers of animals, and therefore are not practical for the routine testing of mutagens. As an alternative to monitoring the pedigree, cells from exposed individuals may be considered for screening for point mutations through the use of an appropriate marker protein. The advantage of such a single-cell screening system (cell specific-locus test) would be that each cell, rather than each individual, could be examined for mutational events.

Single-cell mutation monitoring systems are the ones that detect mutational events in individual cells rather than whole animals. A few single-cell mammalian mutation systems have been developed and are in use in various laboratories. Several others are in various stages of development. The purpose of this book is to bring together such mutation detection methods. Special attention has been paid to include enough experimental details to allow easy

adaptation of the techniques by different investigators. Each chapter is divided into sections comprised of experimental details and sample results.

Each chapter in this book describes a mutation monitoring system that uses one of the easily available cell samples from animals or humans, e.g., red blood cells, lymphocytes, and spermatozoa. The Chapters 1–3 and 9 describe methods based on the use of red blood cells. Six chapters describe methods that use lymphocytes. The last chapter is based on the use of spermatozoa.

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Contents

Somatic-Cell Mutation Monitoring System Based on Human Hemoglobin Mutants

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1. INTRODUCTION

1.1. The Approach

The system described in this chapter was developed as a means of detecting rare red cells, in genetically normal (HbA/HbA) individuals, that are heterozygous for an abnormal hemoglobin. It is assumed, first, that mutations arise spontaneously in human hemopoietic stem cells, as they do in gametal stem cells, and second, that somatic mutations of globin-chain genes do not diminish the viability of affected stem cells. The latter assumption is a reasonable one, since phenotypic expression of such mutations occurs very late in hemopoietic cell differentiation. It is expected that as a result of these stem cell mutations, lines of stem cells containing the mutant globin genes are established and produce erythrocytes heterozygous for structurally abnormal globin chains. Development of appropriate methods of screening blood samples should then permit

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detection and enumeration of red cells that contain an abnormal hemoglobin as a result of somatic mutation in a stem cell.

In principle, an efficient and sensitive method for detecting mutant erythrocytes in genetically normal persons would be of significant value to mutation research, since the study of but a few subjects would be required to assess mutagenic effects of various environmental agents. Similarly, the monitoring of populations for the effects of known mutagens would require longitudinal studies of only a few persons. Since the effects of mutagens on human subjects could be studied directly, the relevance of such studies to human health problems would be apparent. Questions concerning age- or sex-related differences in metabolism or the possibility of polymorphic variation in systems involved in biotransformation of mutagens could be investigated in studies of both populations and families. Finally, a system for scoring somatic mutants of hemoglobin in animal models could be used to correlate frequencies of somatic and gametal mutation.

1.2. Previous Studies

Detection of red cells bearing somatic mutations was first attempted by Atwood[1] and Atwood and Scheinberg.[2,3] They noted that a small proportion (about one in 10^3) of erythrocytes from subjects of blood type AB or A failed to carry the agglutinogen A. Such cells were originally regarded as somatic mutants. On these grounds, Atwood and Scheinberg[2] calculated a somatic mutation rate of 7×10^{-6} per cell division. The mutational origin of the abnormal red cells was subsequently tested by measuring the frequencies of non-A or non-B erythrocytes in AB heterozygotes and BB homozygotes.[4] If the abnormal cells were mutants, their frequency in BB homozygotes should have equaled the square of their frequency in AB heterozygotes. Although BB homozygotes had fewer abnormal cells than did the heterozygous subjects, the frequency of these cells was 50–200 times higher than expected. These findings suggested that it is unlikely that the absence of an A or B agglutinogen from a rare red cell represents the outcome of a somatic-cell mutation.

Sutton[5,6] attempted to relate the presence of red cells with the phenotype characteristic of hereditary persistence of fetal hemoglobin (HPFH; Section 2.2) to the occurrence of somatic mutations. This condition is associated with continuation of synthesis of fetal hemoglobin in all circulating red cells of adult carriers of the abnormal gene. Rare red cells (F cells) that contain fetal hemoglobin are also found in persons who are not carriers of an HPFH determinant. It was postulated that F cells were somatic mutants.[5,6] Results of studies, using sensitive methods, render this a highly unlikely possibility, since F cells constitute from 0.5% to 5.0% of the erythrocytes in every normal adult,[7,8] and their frequencies are elevated in various states, including anemias and hemo-