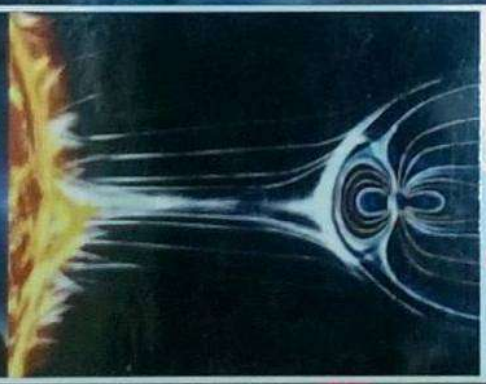
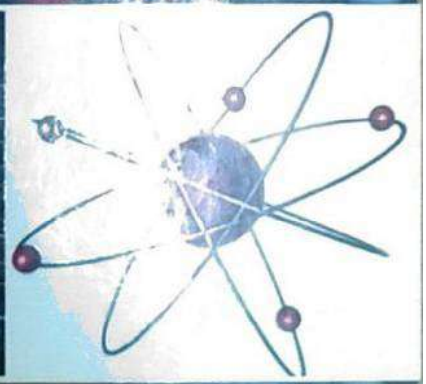
Trends in EXPERIMENTAL BIOLOGY

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EXPERIMENTAL BIOLOGY

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Targeting Metabolic Pathways for Disease Therapy

J. Laskar, S. Nath, S.K. Ghosh, M. Sengupta and Y. Choudhury

INTRODUCTION

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- Anabolic pathways involved in the synthesis and polymerization of simple molecules into complex macromolecules,
- Catabolic pathways involved in degradation of molecules to release energy, and
- Waste disposal pathways which govern elimination of toxic waste.

Core metabolism includes pathways for the synthesis and breakdown of carbohydrates, fatty acids, and amino acids, which are the most vital processes for energy homeostasis and macromolecular synthesis in humans.

Illustrating these pathways and understanding their physiological roles have been among the most fruitful pursuits in biological research. The "Golden Age of Biochemistry" between the 1920s and 1960s defined almost all the metabolic processes responsible for nutrient consumption and energy production in humans as well as in other organisms. These included glycolysis, respiration, the tricarboxylic acid (TCA), urea cycle, glycogen catabolism, oxidative phosphorylation, and the supremacy of ATP in energy transfer reactions and many more. Biochemistry and the analysis of metabolic pathways dominated basic and medically oriented research during these decades, with some 15 Nobel Prizes in either Physiology/Medicine or Chemistry awarded for work related to energy balance or basic metabolic pathways. The driving force behind metabolic research was the realization that metabolic perturbations-often genetically programmed-accompany several

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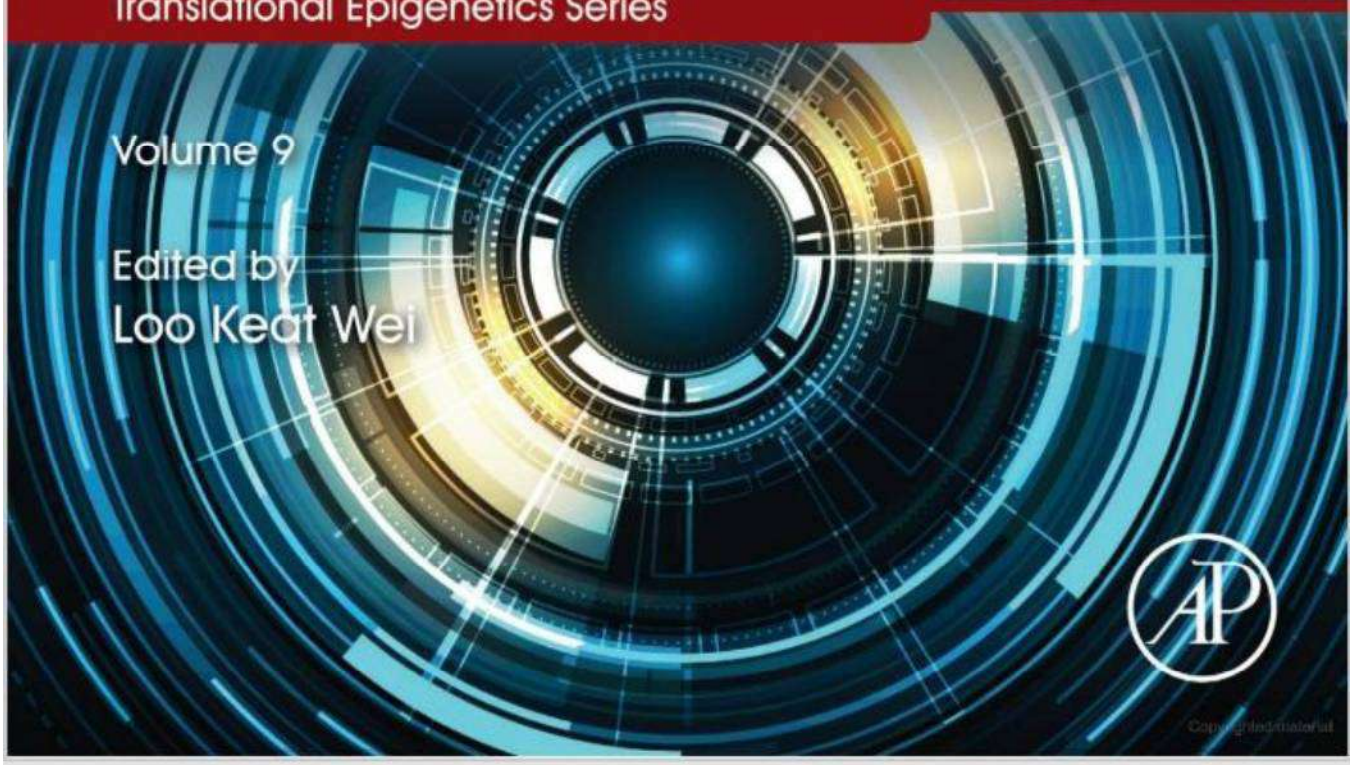

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CHAPTER

EPIGENETIC PROFILING IN HEAD AND NECK CANCER

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Javed Hussain Choudhury[1], Sharbadeb Kundu[1], Fazlur Rahaman Talukdar[2], Ruhina S. Laskar[2], Raima Das[1], Shaheen Laskar[1], Bishal Dhar[1], Manish Kumar[1], Sharad Ghosh[3], Rosy Mondal[4], Yashmin Choudhury[1], Sankar Kumar Ghosh[1,5]

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INTRODUCTION

One of the fundamental questions regarding the diversity of phenotypes within a population is why monozygotic twins or cloned animals can have different phenotypes and disease susceptibility despite their identical DNA sequences; classic genetics is unable to explain these phenomena. However, the concept of epigenetics offers a partial explanation of these phenomena. In 1939, C. H. Waddington introduced "the causal interactions between genes and their products, which bring the phenotype into being." Later on, the term *epigenetics* was described as the study of heritable changes in gene expression without any changes in the DNA sequences. Epigenetic gene patterns play a fundamental role in diverse biological development including embryonic changes, X-chromosome inactivation, and genetic imprinting [1,2]. Unlike genetic changes, epigenetic alterations are reversible, and the key processes involved in epigenetic regulation include DNA methylation, chromatin modification (covalent alteration in core histones), nucleosome positioning, and posttranslational gene expression regulation by noncoding RNAs. Epigenetic changes occur more often than genetic mutation and may persevere for the entire cell life and even for multiple generations. Disruptions of these epigenetic processes can cause aberrant gene expression and function, which may lead to initiation, development, and progression of cancer [3].

Head and neck cancer (HNC) is a broad term that refers to a heterogeneous group of malignancies that arise in the oral cavity, larynx, pharynx, nasal cavity, and paranasal sinuses. Globally, HNC is the sixth most frequent malignancy, accounting for more than 650,000 new cases and 350,000 deaths annually [4]. The development of HNC is a multistep process modulated by genetic, epigenetic, and environmental factors. The environmental risk factors such as tobacco smoking and chewing, in addition to HPV infection, may influence a wide range of genetic and epigenetic alterations that promote genomic and epigenetic instability and endorse tumor development. Epigenetics is a bridge between genotype and phenotype, a phenomenon that changes the ultimate outcome of a genetic locus

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Chapter 1

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Chapter 11

Detection of p16 Promoter Hypermethylation by Methylation-Specific PCR

Javed Hussain Choudhury, Raima Das, Shaheen Laskar, Sharbadeb Kundu, Manish Kumar, Partha Pratim Das, Yashmin Choudhury, Rosy Mondal, and Sankar Kumar Ghosh

Abstract

DNA methylation plays a decisive role in the regulation and control of gene expression. DNA methylation is a covalent modification, in which a methyl group is attached to the 5th carbon of the cytosine ring of a CpG dinucleotide that is located upstream from the promoter region of a gene. Promoter hypermethylation (gain of DNA methylation) of the *p16* gene may cause silencing of gene expression and plays an important role in cancer. Therefore, detection of the methylation status of *p16* gene is an important tool in epigenetic studies of various human cancers. The methylation-specific PCR (MSP) is the most commonly used technique for studying DNA methylation. This technique is based on bisulfite modification of DNA, which converts unmethylated cytosine (C) into uracil (U) and leaving methylated cytosine (C^m) unchanged. Here we describe the bisulfite modification of DNA samples and detection of promoter methylation of *p16* gene from bisulfite-treated DNA using MSP. In MSP, modified DNA samples are subjected to PCR amplification using methylated and unmethylated specific primers for the *p16* gene separately. The PCR amplified products are then analyzed in a 2.5–3% agarose gel containing ethidium bromide. The PCR amplified band generated by specific sets of primers is used to determine the methylation status of the *p16* gene.

Key words DNA methylation, *p16* gene hypermethylation, Bisulfite modification, Specific primers, Methylation-specific PCR, Agarose gel electrophoresis

1 Introduction

DNA methylation plays a crucial role in the regulation and control of gene expression. DNA methylation is a covalent modification, in which a methyl group is attached to the 5th carbon of the cytosine ring of a CpG dinucleotide (at CpG Islands) by the enzyme DNA methyltransferases (DNMTs). The CpG dinucleotide is located upstream from the promoter region of a gene [1]. Promoter hypermethylation (gain of DNA methylation) can cause silencing of tumour suppressor's pathway genes (such as *p16*, *p53*, *DAPK*, *ECAD*, and *RASSF1A*) in various human cancers. Therefore,

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