Chapter 7
Epigenetic Regulation of Connexins

Besides regulation of connexin expression by the direct binding of transcription factors to their regulatory elements, various other epigenetic mechanisms are involved in the expression of connexin gene. Regulation at the epigenetic level is a prerequisite for other regulatory mechanisms to function. Epigenetic processes play an important role in the regulation of connexin gene expression. This type of regulation has important physiological significance, and any disruption in this type of regulation has severe consequences on the cell physiology. One such example is the role of epigenetic factors on the cell growth and proliferation. Connexins are known to regulate cell proliferation, and it is well established that they possess tumour suppressor function. Thus, it is anticipated that the connexins are the target of various epigenetic regulatory mechanisms. Involvement of various epigenetic mechanisms in connexin expression and function is discussed below.

7.1 Histone Acetylation/Deacetylation

Like other eukaryotic genes, connexin genes are present on various chromosomes and thus are warped in protein–DNA complex. Histones form the important protein components of these complexes. Histones are subject to post-translational modification by enzymes, and these modifications include methylation, citrullination, acetylation, phosphorylation, SUMOylation, ubiquitination, and ADP-ribosylation. The main function of these modifications is to affect the gene expression by remodelling the chromatin structure of a particular gene. For the transcription of connexin genes, the chromatin structure needs to be modified, and histones are the prime targets of these modifications. Studies have demonstrated that the acetylation/deacetylation constitutes an important mechanism of the connexin gene regulation. For example, the use of various histone deacetylase inhibitor results in the increased Cx43 transcription. The increased transcription of Cx43 is attributed due to more acetylation of H3 and H4 histones of the chromatin near the promoter region of Cx43. In one such study,

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it was shown that the treatment of prostate cancer cells with trichostatin A (TSA) results in the increased acetylation of histones in the Cx43 promoter. The increased acetylation was found due to the recruitment of p300/CREB-binding protein, a transcriptional coactivator displaying histone acetyltransferase (HAT) activity, to the promoter region of Cx43. Because of the chromatin remodelling, the binding of transcription factors AP-1 and Sp1 to the Cx43 gene promoter is enhanced manifold with the concomitant increase in Cx43 transcription.

7.2 DNA Methylation

DNA methylation is one of the important epigenetic mechanisms of controlling the expression of genes. DNA methylation dynamics are mostly associated with the promoter regions of genes. Promoter methylation occurs mostly at the CCGG sequences known as CpG islands. In this context, hypermethylation of promoter regions results in the silencing of the gene expression. DNA methylation of promoter regions of connexins has been well studied, and most of these are frequently studied in a clinical context. Downregulation of connexin expression has been associated with the hypermethylation of their promoter. Cx26, Cx32, and Cx43 promoter elements have been found hypermethylated in various malignant tissues like cultured human lung cancer cells, primary human renal carcinoma cells, cultured human oesophageal cancer cells, etc. Similarly, in liver carcinogenesis, Cx26 expression is downregulated, and this has been found to have strong correlation with the hypermethylation state of its promoter. The mechanism responsible for the connexin gene silencing and hypermethylation is not well understood. Many factors have been attributed as a link between DNA hypermethylation and impairment of connexin expression. It has been shown that hypermethylation results in the aberrant binding or recruitment of transcription factors to the promoter elements. For example, Cx43 promoter has been found hypermethylated in human primary non-small cell lung carcinoma, and this has been related with the reduced binding of AP1 transcription factor. Moreover, the Sp1 binding site overlaps with the CpG islands, which are the prime targets of methylation. In addition, Sp1 is a ubiquitous transcription factor involved in the expression of various connexin genes. Thus, it may be construed that hypermethylation will result in the aberrant binding of Sp1 transcription factor. In fact, Sp1 binding sites of the Cx26 gene promoter and the Cx32 gene promoter in cultured and primary human breast cancer cells have been found hypermethylated with the concomitant decrease in the expression of these connexins.