with age.[154] In contrast, some loci have been found hypermethylated with age (*e.g.* estrogen receptor, interferon γ, insulin-like growth factor II, promoters of tumor-suppressor genes such as lysyl oxidase (*LOX*), *p16INK4a*, runt-related transcription factor 3 (*RUNX3*), and TPA-inducible gene 1 (*TIG1*)).[153] Xu and Taylor[155] analyzed 1006 blood DNA samples of women aged 35 to 76 from the Sister Study, and found that 7694 (28%) of the 27 578 CpGs assayed were associated with age, confirming the existence of at least 749 "high-confidence" age-related CpG (arCpGs) sites in normal blood. These age-related changes are largely concordant in a broad variety of normal tissues, and a significantly higher proportion (71–91%) than expected of increasingly-methylated arCpGs (IM-arCpGs) were over-methylated in a wide variety of tumor types. IM-arCpGs sites occurred almost exclusively at CpG islands and were disproportionately marked with the repressive H3K27me3 histone modification. These findings suggest that as cells acquire methylation at age-related sites they have a lower threshold for malignant transformation that may explain in part the increase in cancer incidence with age.

McClay *et al.*[156] performed a methylome-wide association study of aging in whole blood DNA from 718 individuals, aged 25–92 years. They sequenced the methyl-CpG-enriched genomic DNA fraction, averaging 67.3 million reads per subject, to obtain methylation measurements for the ~27 million autosomal CpGs in the human genome, and adaptively combined methylation measures for neighboring, highly correlated CpGs into 4 344 016 CpG blocks for association testing. Eleven age-associated differentially methylated regions (DMRs) passed Bonferroni correction. 42 of 70 selected DMRs showed hypomethylation and 28 showed hypermethylation with age. Hypermethylated DMRs were more likely to overlap with CpG islands and shores. Hypomethylated DMRs were more likely to be in regions associated with polycomb/regulatory proteins (EZH2) or histone modifications H3K27ac, H3K4m1, H3K4m2, H3K4m3 and H3K9ac. Among genes implicated by the top DMRs were protocadherins, homeobox genes, mitogen-activated protein kinases (MAPKs), ryanodine receptors, and genes with potential relevance for age-related disease.

The absolute levels of 5-hydroxymethylcytosine (hmC), 5-formylcytosine (fC) and 5-methylcytosine (mC) vary in human brain tissues at various ages. For hmC, an initial steady increase is observed, which levels off with age to a final steady-state value of 1.2%. This level is nearly twice as high as in mouse cerebral cortex. fC declines rapidly with age during early developmental stages. While hmC is a stable epigenetic mark, fC is more likely an intermediate of active DNA demethylation during early brain development. The trends in global cytosine modification dynamics during the lifespan are conserved between humans and mice and show similar patterns in different organs.[157]

5.4.1.2 Histone Modifications

Histone modifications are also observed with aging. Histone acetylation decreases and phosphorylation increases with age.[158] H4K20me and H3K36me3 decrease in the brain of old senescence-accelerated-prone