5 Summary

Epigenetical inactivation of the *RASSF1A* tumor suppressor gene was frequently detected in cancer. However, the mechanisms of aberrant DNA methylation in the *RASSF1A* CpG island are unknown. In the present study, four *Sp1* sites in the *RASSF1A* promoter were characterized; and the functional relationship between DNA methylation, histone modification, *Sp1* binding and *RASSF1A* expression was examined in proliferating human mammary epithelial cells. With increasing passages of HMECs, the transcription of *RASSF1A* was dramatically silenced and this was associated with deacetylation and lysine 9 trimethylation of histone H3 and spreading DNA methylation from upstream and downstream into promoter. The *RASSF1A* CpG island in HMECs, which had overcome a stress-associated senescence barrier, was characterized by *de novo* DNA methylation and an elevated level of trimethylated histone H3 lysine 9. The binding of *Sp1* to the *RASSF1A* promoter was impaired in these cells. The present data suggest that the chromatin inactivation occurs in the same time window as gene inactivation and may precede the *de novo* DNA methylation. Moreover, present results indicate that the *Sp1* binding is mediated by chromatin state and not by DNA methylation. In summary, progressive histone inactivation, spreading of DNA methylation and inactivation of the *Sp1* binding were observed in the *RASSF1A* promoter during senescence of HMECs and this system may serve as a model for the epigenetical inactivation of the tumor suppressor gene in carcinogenesis.