Abstract

Promyelocytic leukemia protein (PML) is a tumour suppressor which is frequently downregulated in human tumours. PML plays a role in many cellular processes including DNA damage response, senescence and apoptosis and is mainly localized in special structures called PML nuclear bodies (PML NBs). The nucleolus is a key nuclear compartment, where transcription of ribosomal DNA and biogenesis of ribosomes take place. The nucleolus is also called a stress sensor because of its role, for instance, in stabilization of tumour suppressor p53. Localization of PML to the nucleolar periphery appears to be prominent after disturbance of nucleolar functions – for example inhibition of rRNA transcription or processing. Thus the relationship between the nucleolus and PML nuclear bodies may be important for cellular response to stress. However, the role of PML nucleolar associations in nucleolar function including mechanism of formation of these structures remain unclear.

Here we characterised PML nucleolar structures and mechanism of their formation. We showed that formation of PML nucleolar structures is not caused by replication stress, is not dependent on any specific phase of cell cycle and is not caused by DNA damage response but is induced by topological stress due to inhibition of toposiomerase function. When deciphering the mechanism of induction of PML expression in cells exposed to DNA topoisomerase inhibition, we found that the PML expression is mediated by transcription factor p53. Regarding mechanisms of formation of PML nucleolar structures, both relocalization of PML nuclear bodies and their de novo formation on the nucleolar surface were observed. The PML structures associated at the nucleolar border possessed similar protein composition as 'regular' PML NBs. We described changes of nucleolar structure during DNA topoisomerase inhibition including relocalization of some nucleolar proteins in spatial relationship to PML nucleolar associations. Furthermore, we described dynamics and mutual transformations of PML nucleolar structures including relocalization of nucleolar material into PML structures derived from nucleoli. The presence of only some proteins in these structures indicated that the sequestration of nucleolar content into these structures is a selective process. Furthermore we found a crucial role of PML isoforms I and IV in formation of PML nucleolar structures. We attempted to identify a PML domain responsible for nucleolar localization and we showed that previously described PML ‘nucleolar localization sequences’ are not prerequisite for relocalization of PML to the nucleolus during stress response.

In this work we confirmed and extended some previous findings and gained new data about PML nucleolar structures that can be helpful for understanding of the complex process of PML targeting to the nucleolus and its function. Since we found that the formation of the PML nucleolar structures is specific only to human normal (non-cancerous) cells, our findings may have also important implication for cancer biology.

Keywords: PML, nucleolus, PML nucleolar structures, genotoxic stress, DNA damage response, nucleolar stress, p53, cell cycle