Selective binding of TIMPs to chromosomes

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We reported earlier that TIMP-1 accumulated in the nuclei of human gingival fibroblasts in a cell cycle-dependent manner (JCS 111 1147 1998). However, tumor cells generally proliferate faster than normal cells and have no clear cell cycle. The majority of their nuclei always showed TIMP-1 positive staining. Furthermore, the chromosomes in the M phase cells showed strongly positive TIMP-1 staining, suggesting specific binding between TIMP-1 and some component(s) in the chromosomes. In this present study, we examined other members of the TIMP family; i.e., TIMP-2, -3 and -4. The nuclei of HeLa and MG63 cells were positive for these three TIMPs when analyzed by the ABC immunohistochemical method. The staining profile of TIMP family members on chromosomes was, however, a little different from that in nuclei; i.e., both TIMP-3 and -4 showed similar staining as TIMP-1, Whereas most chromosomes were negative for TIMP-2, suggesting that TIMP-2 might not bind to chromosomes or do so only very weakly. After treating HeLa cells in culture with colchichine, we collected the cells in M phase. Then the chromosomes were isolated from the cells by using either the digitonin-polyamine discharge (DPD) or Ficoll fractionation method. The chromosome fraction prepared by rather harsh DPD method showed only TIMP-1 in a Western blot, but the fraction prepared by the rather mild Ficoll fractionation method showed TIMP-3 and -4 besides TIMP-1, even though there were differences in their staining intensity. TIMP-2 was, however, not detected in either chromosome fraction. In summary, both immunohistochemical staining of cells in M phase and Western blot analysis of chromosome fractions prepared by two different methods showed the presence of selective binding of four TIMPs to chromosomes.