The interphase microtubule damage checkpoint defines an S-phase commitment point and does not require p21waf-1

Charlie R. Mantel, Stephen E. Braun, Younghee Lee, Young-June Kim, and Hal E. Broxmeyer

Cell cycle checkpoints ensure orderly progression of events during cell division. A microtubule damage (MTD)-induced checkpoint has been described in G1 phase of the cell cycle (G1 MTC) for which little is known. The present study shows that the G1 MTC is intact in activated T lymphocytes from mice with the p21 waf-1 gene deleted. However, p21waf-1 gene deletion does affect the ratio of cells that arrest at the G1 MTC and the spindle checkpoint after MTD. The G1 MTC arrests T lymphocytes in G1 prior to cdc2 up-regulation and prior to G1 arrest by p21waf-1. Once cells have progressed past the G1 MTC, they are committed to chromosome replication and metaphase progression, even with extreme MTD. The G1 MTC is also present in a human myeloid cell line deficient in p21waf-1 gene expression. The p21-independent G1 MTC may be important in cellular responses to MTD such as those induced by drugs used to treat cancer. (Blood. 2001;97:1505-1507)
compensation have been described. Statistical comparisons used the Student t test. Experiments were performed at least 3 times.

Results and discussion

Figure 1 shows cdc2 expression in activated murine T lymphocytes as a function of DNA content. The cdc2 content is up-regulated in G1 phase. There are at least 2 separate populations of G1 cells with respect to cdc2 levels, G1a and G1b. Cells enter S and G2/M phases with very little further increase in cdc2 expression. After mitosis, cdc2 is degraded and the daughter cells return to G1 phase with little or no cdc2. A 3-fold shift in the relative proportions of G1a and G1b cells is observed in the cells with the p21 gene deleted. This is consistent with the proposed role of p21 in G1 phase progression and “threshold” events as reported. After treatment with the MT depolymerizing agent, nocodazole, the G1b population could not be observed in wild-type or p21 knockout mouse cells (Figure 1C,D). However, the relative proportion of G2/M phase cells was higher in the p21 knockout cells after treatment compared to wild-type cells. There was also an increase in the number of 8N cells in the p21 knockout cultures after nocodazole treatment compared to wild-type cells. Treatment with taxol resulted in a similar response (not shown). We interpret this as indicating that G1b cells are arresting after MTD at a point in G1 phase before cdc2 expression is up-regulated. Once cells have progressed in the cell cycle past this point, and cdc2 expression is turned on, subsequent MTD no longer arrests the cells in G1, but they progress through S and G2 phases to arrest at the SAC in mitosis. Thus, the point of cdc2 up-regulation defines the point of passage of the G1 MTC when cells become committed to DNA replication, even in the presence of MTD. The absence of p21 does not appear to influence G1 arrest by the G1 MTC. However, the temporal effect of p21 deletion on the proportions of cells at G1a versus G1b leads to fewer cells that arrest at the G1 MTC after MTD, and leads to the increased proportion of p21 knockout cells arrested in mitosis as reported.4-7

An identical experiment was performed on p21-deficient human myeloid cell line, MO7e, after MTD. Scatter density diagrams of bivariate cell cycle analysis of vector control cells (LXSN; A,C) and p21 antisense (AS21; B,D) are shown. Day 3 cell cultures were treated for 24 hours with 15 μg/mL nocodazole (G1) or control diluent (A,B). The 2N (G0/G1) and 4N (G2/M) DNA content is indicated along with the percentages of cells in different cell cycle phases. Data are representative of at least 6 separate experiments done in triplicate.

An interesting finding is the appearance of a population of 4N...
human cells with low (Figure 2C) or negative (Figure 2D) cdc2 content that is not observed in murine cells. This population could represent p21-deficient cells that have prematurely exited mitosis without cytokinesis and thus have escaped cell cycle arrest induced by SAC activation, suggesting a difference between human and murine responses to MTD. Embryonic cells from p21−/− mice have been reported to have an intact SAC response, but do fail to prevent re-replication of DNA events consistent with our analysis. On the other hand, loss of p53 in a human tumor cell line is reported to be without effect on nocodazole arrest. Rodent cells are known to have “leaky” or missing cell cycle checkpoints compared to human cells. Also, somatic cells may have different or additional checkpoints compared to embryonic cells. Therefore, cell type and species differences in response to MTD are possible. These issues are currently under investigation.

Questions yet to be answered are: What is the purpose of the G1MTC and what is the nature of the MTD sensing mechanism? The sensing apparatus of the SAC is believed to reside at the kinetochore, and tension on 2 juxtaposed MT attachment points is believed to be the key mecha-nochemical process that senses correct chromosome-spindle alignment and sends a “go” signal for anaphase initiation and chromosome congression.14 Because the centrosome is the MT organizing center of interphase cells, and because this organelle must duplicate and separate in G1 phase ultimately to become the poles of the mitotic spindle, we speculate that the G1MTC defines a cell cycle checkpoint ensuring proper centriole duplication and separation. Figure 3 illustrates the “location” of the G1MTC in relation to the SAC, the centrosome cycle, and the DNA cycle. The relationship between the G1MTC and the restriction point, or other cell cycle commitment points or checkpoints, remains to be determined. However, the existence of an interphase MTD checkpoint has significant implications in treatment strategies of cancer by drugs that exert selective toxicity on cancer cells by interfering with MT dynamics.

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References

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