

Abnormal Centrosome Amplification and Aurora-A Activation in p53-deficient Cells

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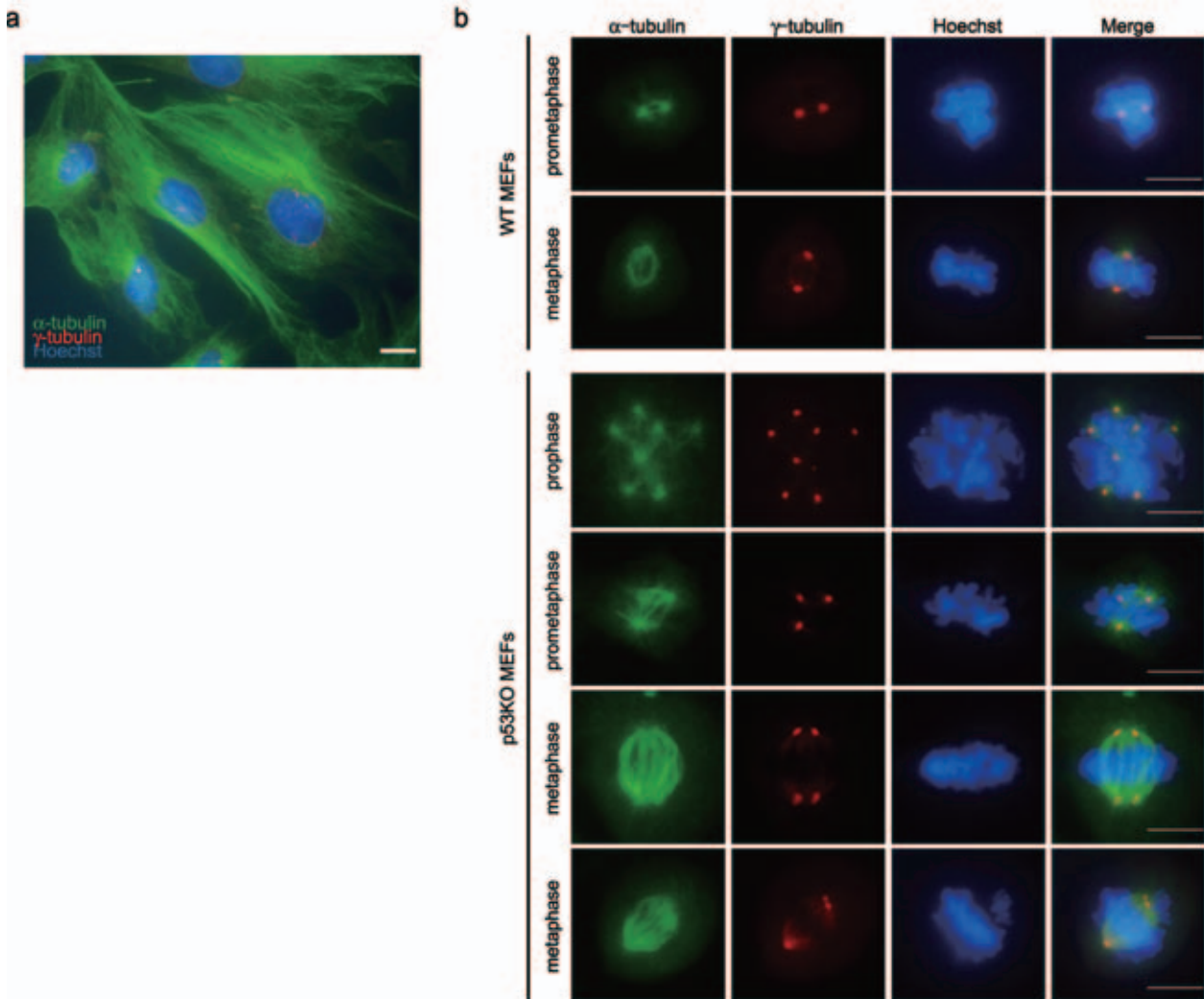


Fig. 1

The *p53* tumor-suppressor gene is the most frequent target of genetic alterations in human cancer. Numerous studies have demonstrated that the loss of *p53* function results in genetic instability, such as mutation, chromosomal translocation, and aneuploidy. It has been shown that the loss of *p53* function leads to an abnormal centrosome amplification because of the deregulation of the centrosome duplication cycle. As shown in **Figure 1a**, centrosome hyperamplification (stained with anti- γ -tubulin antibody; red) was frequently observed in *p53*-deficient (*p53*^{-/-}) mouse embryonic fibroblasts (MEFs) at interphase. During mitosis, centrosomes form spindle poles and play a vital role in bipolar spindle formation and accurate chromosomal segregation (**Fig. 1b**). In contrast, the mitotic *p53*^{-/-} MEFs frequently displayed aberrant spindles, organized by multiple copies of

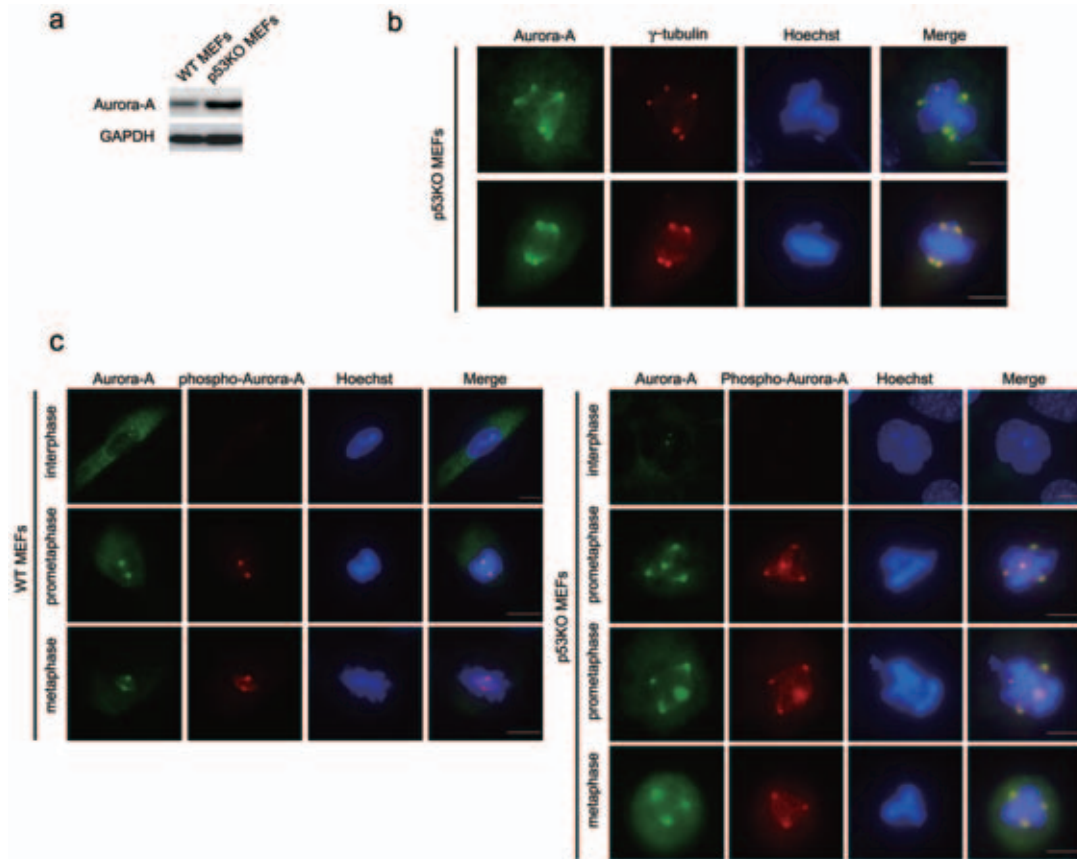


Fig. 2

centrosomes (**Fig. 1b**).

Aurora-A is a mitotic kinase that localizes to the centrosome and the bipolar mitotic spindle poles. It has been also shown that overexpression of Aurora-A induces centrosome amplification in cultured cells. We analyzed Aurora-A protein levels and found that those in $p53^{-/-}$ MEFs were higher than those in wild type (WT) MEFs (**Fig. 2a**). Moreover, Aurora-A was found to localize to abnormally amplified centrosomes in $p53^{-/-}$ MEFs (**Fig. 2b**). We also found that Aurora-A is activated in abnormally amplified centrosomes in $p53^{-/-}$ MEFs (**Fig. 2c**). Therefore, these results suggest that activated Aurora-A caused by the loss of p53, “the guardian of the genome.” is involved in abnormal centrosome amplification.

Fig. 1 (a) $p53^{-/-}$ MEFs at interphase were coimmunostained with anti- α -tubulin antibody (green: Cell Signaling Technology) and anti- γ -tubulin antibody (red: Sigma). DNA was stained with Hoechst 33342 (blue: Invitrogen). Scale bars: 10 μ m.

(b) Mitotic WT or $p53^{-/-}$ MEFs coimmunostained with anti- α -tubulin antibody (green) and anti- γ -tubulin antibody (red). DNA was stained with Hoechst 33342 (blue). Scale bars: 10 μ m.

Fig. 2 (a) Aurora-A protein levels in WT and $p53^{-/-}$ MEFs were determined by immunoblot analysis. (b) The mitotic $p53^{-/-}$ MEFs were coimmunostained with Aurora-A (green: Novus Biologicals, Inc) and anti- γ -tubulin antibody (red). DNA was stained with Hoechst 33342 (blue). Scale bars: 10 μ m. (c) Mitotic WT or $p53^{-/-}$ MEFs were coimmunostained with anti-Aurora-A antibody (green) and anti-phosphorylated Aurora-A antibody (red: This antibody was a gift from Dr. Urano, Nagoya University Graduate School of Medicine). DNA was stained with Hoechst 33342 (blue). Scale bars: 10 μ m.