Regulation of cell behaviors by using nanotopography has been widely studied. To facilitate cell adhesion, nanotopography is usually coated with adhesive proteins such as fibronectin (FN). However, current understanding regarding the role of the physical and biochemical cues in cell regulation is still limited. To delineate the physical and biochemical regulation of stem cell behaviors, we designed and fabricated nanogratings and rectangular nanopillars on polystyrene (PS) substrates, and then stamped nanopatterned FN including nanogratings or array of rectangles on the surfaces of PS nanotopographies and flat substrates to decouple the nanotopographical and biochemical cues. Our results showed that both nanotopography and FN patterns could influence the cell elongation, YAP intracellular localization and lamin a/c (LAMAC) expression of human mesenchymal stem cells (hMSCs). Physical nanogratings significantly enhanced the cell/nucleus elongation, but decreased the YAP nuclear localization and LAMAC expression of hMSCs, as compared to those of the cells on PS flat surfaces or rectangular nanopillars with the identical FN patterns. FN nanorectangles attenuated the nanogratings effects by reducing cell elongation and enhancing YAP nuclear localization and LAMAC expression. However, the cellular/nuclear area, proliferation and differentiation of hMSCs were dominantly controlled by physical nanotopography. The nanogratings were further found to induce nanoscale curvature of the nuclei at the basal side, which was related to the LAMAC expression. Collectively, our study indicated that both physical nanotopography and biochemical cues played roles in stem cell regulation, while the physical nanotopography had more profound influences than the biochemical cues.