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Isobutyrylshikonin Elicits Reactive Oxygen Species-Mediated Necroptosis, not Apoptosis

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SUMMARY*. Isobutyrylshikonin (IBS), purified from the root of *Lithospermum erythrorhizon* Sieb. et Zucc. (Boraginaceae), exhibits various pharmacological properties. However, the anti-cancer activity of IBS has not been fully elucidated. In the current study, we investigated whether IBS elicits cell death via necroptosis in MCF-7 and MDA-MB-231 cancer cells. Our data showed that IBS induces high propidium iodide $(PI)^+$ and annexin-V- populations at the early stage of cell death in both cancer cell types, and high PI^+ and annexin-V^+ cell populations at the late stage, suggesting that IBS promotes necroptosis. Moreover, specific inhibitors of caspases, including a caspase-3 inhibitor, Z-DEVD-FMK, a caspase-8 inhibitor, Z-IETD-FMK, a caspase-9 inhibitor, z-LEHD- FMK, and a pan caspase inhibitor, Z-VAD-FMK, did not downregulate IBS-induced cell death in the cancer cells, indicating that IBS-induced cell death is not apoptotic. Treatment with necrostatin-1 (NS-1), a potent and selective inhibitor of necroptosis, inhibited IBS-mediated cell death, and moreover, attenuated the percentage of cells at the sub-G1 phase after IBS treatment. Fluorescence analysis using 2'7'-Dichlorofluorescin diacetate (DCFDA) showed that IBS cumulatively increased reactive oxygen species (ROS) production, and demonstrated that the ROS inhibitors, N-acetylcysteine (NAC) and glutathione (GSH) completely reversed IBS-induced cell death. Taken together, these results indicate that IBS significantly induces cell death via necroptosis in MCF-7 and MDA-MB-231 cancer cells by increasing ROS generation, suggesting that IBS is a potential candidate for use in apoptosis-resistant cancers.

RESUMEN. Isobutyrylshikonina (IBS), purificada de la raíz de *Lithospermum erythrorhizon* Sieb. et Zucc. (Boraginaceae), exhibe diversas propiedades farmacológicas. Sin embargo, la actividad anticancerígena de IBS no se ha aclarado completamente. En el estudio actual, investigamos si IBS provoca muerte celular por necroptosis en células cancerosas MCF-7 y MDA-MB-231. Nuestros datos mostraron que IBS induce poblaciones con alto yoduro de propidio $(PI)^+$ y anexina-V en la etapa temprana de la muerte celular en ambos tipos de células cancerosas, y poblaciones de células PI^+ y anexina-V altas en la etapa tardía, lo que sugiere que el IBS promueve necroptosis. Además, los inhibidores específicos de las caspasas, incluyendo un inhibidor de la caspasa-3, Z-DEVD-FMK, un inhibidor de la caspasa-9, Z-IETD-FMK, un inhibidor de la caspasa-8, Z-LEHD-FMK y un inhibidor de la caspasa, Z-VAD-FMK. no regularon la reducción de la muerte celular inducida por IBS en las células cancerosas, lo que indica que la muerte celular inducida por IBS no es apoptótica. El tratamiento con necrostatin-1 (NS-1), un inhibidor potente y selectivo de la necroptosis, inhibió la muerte celular mediada por IBS y, además, atenuó el porcentaje de células en la fase sub-G1 después del tratamiento con IBS. El análisis de fluorescencia con diacetato de 2'7'-diclorofluorescina (DCFDA) mostró que IBS aumentó de forma acumulativa la producción de especies reactivas de oxígeno (ROS) y demostró que los inhibidores de la ROS, la N-acetilcisteína (NAC) y el glutatión (GSH) revirtieron completamente la muerte celular inducida por IBS. En conjunto, estos resultados indican que IBS induce significativamente la muerte celular por necroptosis en las células cancerosas MCF-7 y MDA-MB-231 al aumentar la generación de ROS, lo que sugiere que el IBS es un posible candidato para su uso en los cánceres resistentes a la apoptosis.

KEY WORDS: isobutyrylshikonin, necroptosis, reactive oxygen species.

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