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Nucleus or cytoplasm? The mysterious case of SIRT1's subcellular localization

Wenlong Bai[a] and Xiaohong Zhang[b]

[a]Department of Pathology and Cell Biology, Morsani College of Medicine, University of South Florida, Tampa, FL, USA; [b]Department of Oncology, Karmanos Cancer Institute, Detroit, MI, USA

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Sirtuins are NAD^+-dependent class III histone deacetylases (HDACs) which deacetylate core histones and non-histone proteins.[1] There are a total of 7 members, namely, SIRT1, SIRT2, SIRT3, SIRT4, SIRT5, SIRT6, and SIRT7 in the Sirtuin family. As the founding member, SIRT1 has attracted the most attention and has been extensively studied.[2] Increasing evidence suggests that SIRT1 plays an important role in a wide range of biological and pathological processes, including metabolism, aging, genome stability, cell migration and invasion, metastasis, DNA damage and repair, and cancer.

The Janus face of SIRT1 in cancer is perplexing and controversial.[3,4] Some lines of evidence have suggested that SIRT1 is a tumor suppressor. For example, SIRT1 maintains genomic stability and inhibits pro-inflammatory signaling that is often aberrantly activated in cancer cells. However, other lines of evidence indicate that SIRT1 is a tumor promoter. SIRT1 has been found to be overexpressed in some cancers, such as ovary, liver, breast, stomach, and pancreas and exhibits oncogenic functions. It has been proposed that subcellular localization may account for the dual roles of SIRT1 in normal versus cancer cells.[4] SIRT1 may target its nuclear substrates to exert its tumor suppressor function and target its cytoplasmic substrates to exert its tumor promoter function. Some reports have shown that SIRT1 is predominantly localized in the nucleus in normal cells, but predominantly localized in the cytoplasm in cancer cells[5,6] – a finding consistent with this hypothesis. However, the cytoplasmic localization of SIRT1 in cancer and transformed cells remain controversial. When different approaches, such as cell fractionation and immunofluorescence, were utilized to determine the subcellular localization of SIRT1, the results were often inconsistent. For instance, cell fractionation in most cases showed that SIRT1 is in the cytoplasm in cancer cells[6] while immunostaining usually gave a diverse result depends on the antibodies and protocols were used.[6]

In a recent issue of *Cell Cycle*, Sun and Fang from H. Lee Moffitt Cancer Center & Research Institute have revealed the reasons behind the above controversy.[7] To determine whether the antibodies used for immunostaining are specific for SIRT1, they knocked down SIRT1 in 293T and PC3 cells and performed the immunofluorescence staining analysis. They found that instead of detecting the diminishing signal in the knockdown cells, one of the antibodies detected the same SIRT1 signal in the cytoplasm as that in the control cells, suggesting that this antibody cross-reacted with non-specific proteins. Therefore, the inconsistency of the immunostaining results is attributed to the cross activity of some anti-SIRT1 antibodies.

Next, they discovered that conventional cell fractionation often results in a severe leaking of SIRT1 into the cytoplasm, which is mainly caused by hypotonic dwelling and loss of the cytoplasmic macromolecular crowding effect during the isolation of the nuclei. Sun and Fang developed an improved fractionation method using inert polymers, like Ficoll, containing solution for nuclei isolation. Using this method, they observed the predicated localization of the NLS or NES mutant of SIRT1 and confirmed the results with live cell imaging of GFP-tagged SIRT1 wild-type and SIRT1 NLS or NES mutant. Using this approach, they showed that SIRT1 predominantly resides in the nucleus in all cancer cells tested, such as DU145, PC3, U2OS, MDA-MB-453, and MDA-MB-468. The nuclear localization of SIRT1 was further validated by immunostaining and, for some cells, live cell imaging. They concluded that SIRT1 is predominantly localized in the nucleus and that the modified approach accurately represents the subcellular localization of SIRT1 and is suitable for its translocation study. Intriguingly, using the conventional method, SIRT1 persists in the nucleus of normal cells, such as human fetal lung fibroblast IMR-90. This observation raised the possibility that cancer cells may possess a mechanism by which SIRT1 is prone to be leaked out to the cytoplasm. Further studies along this line will reveal the novel function of SIRT1 in cancer cells. Overall, with the improved Ficoll-digitonin fractionation approach provided in their study, the subcellular localization of SIRT1 in cancer cells can be precisely examined, which will facilitate investigation in the role of SIRT1 in cancer.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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CONTACT Xiaohong Zhang zhangx@karmanos.org Department of Oncology, Karmanos Cancer Institute, 4100 John R. St., Detroit, MI 48201, USA.
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