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COMMENTARY

The Role of SIRT1 in Cancer

The Saga Continues

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In recent years, the importance of histone deacetylases in cancer development has become increasingly apparent. Many nonhistone protein targets of histone deacetylases have been identified as tumor suppressors or promoters, and aberrant expression and regulation of histone deacetylases therefore has been shown to have either oncogenic or protective consequences.[1] SIRT1, a member of the NAD^+-dependent class III histone deacetylases known as the Sirtuins, has been shown to play a role in not only cancer, but also a number of other physiological processes and health conditions, including metabolic disease, inflammation, neurodegeneration, and cardiovascular disease.[2,3] The role of SIRT1 in cancer remains controversial as many studies have suggested a tumor promoter function of SIRT1, whereas a number of other studies have shown a tumor suppressor role of this sirtuin.[4]

In this issue of *The American Journal of Pathology*, Di Sante et al[5] weigh in on this controversy with compelling data supporting the status of SIRT1 as a tumor suppressor in prostate cancer. They used a *Sirt1*$^{-/-}$ mouse model and fibroblasts to demonstrate a protective role of SIRT1 against the induction of prostate intraepithelial neoplasia, increased cellular proliferation, and accompanied mitophagy. In addition, they demonstrate that SIRT1 inhibits reactive oxygen species production and induces superoxide dismutase 2 activity, and that expression of the tumor suppressor TP63 increases with the loss of SIRT1. Here, we discuss the implications of these findings in the context of the current research regarding the role of SIRT1 as a tumor suppressor or promoter.

SIRT1 as an Oncogene or Tumor Suppressor in Cancer

An overview of the available literature shows that SIRT1 is overexpressed in a variety of human cancer cell lines and tissues.[4] This includes several studies in prostate cancer, which are in agreement with the findings of Di Sante et al.[5] Furthermore, chemical inhibition of SIRT1 in prostate cancer has been shown to reduce cellular growth, viability, and chemoresistance, and a recent analysis of prostate cancer tissues showed SIRT1 to be a reliable biomarker of disease recurrence.[6] It would be logical to conclude from these findings that SIRT1 has a tumor promoter function in prostate cancer. However, Di Sante et al[5] have found that in prostate cancer patients, a low level of SIRT1 is associated with decreased recurrence-free survival, supporting a tumor suppressor function of SIRT1 in prostate cancer. The data in *Sirt1*$^{-/-}$ mice also support the tumor suppressor role for SIRT1, which could suggest that the increased levels of SIRT1 in prostate cancer tissues are a response to the cancer rather than a cause. However, this does not explain why chemical inhibition of SIRT1 has been shown to have tumor suppressive effects, thus leading to seemingly contradictory theories.

One possibility for this contradiction could be found in the species being studied. The studies referenced in which chemical inhibition of SIRT1 resulted in tumor suppressive effects were conducted in human prostate cancer cell lines.[4] However, Di Sante et al[5] have used murine cells and *Sirt1*$^{-/-}$ mice for their experiments. Similar results have previously been shown in mouse models of other cancers, which could signify a difference in SIRT1 behavior between mice and humans.[4] Alternatively, *Sirt1*$^{-/-}$ mice are also

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embryonic lethal in many backgrounds, and those that are not frequently have developmental abnormalities such as smaller body size, infertility, and autoimmune deficiencies. It cannot be ruled out that unintended consequences of the *Sirt1*$^{-/-}$ genotype could be contributing to the tumor suppressive phenotype. However, several studies have shown that the overexpression of SIRT1 alone does not increase tumor formation in mice, indicating that either *Sirt1* is not an oncogene, or that additional contributing factors are necessary for the oncogenic function of SIRT1.[5]

Mechanisms That Direct SIRT1 toward Oncogenic or Tumor Suppressor Status

SIRT1 impacts a diverse variety of biological activities, with >50 nonhistone protein targets currently identified.[4] Some of the more noteworthy targets relevant to carcinogenesis are those that function in the DNA damage response, autophagy, cellular metabolism, and cell survival under stress.[4] The role of SIRT1 in these systems is complex, in part due to regulatory feedback loops which can cause different activity levels of SIRT1 to promote different cellular effects. For example, the tumor suppressor TP53, which is a homolog of the protein TP63 analyzed by Di Sante et al,[5] is involved in one such feedback loop with SIRT1. TP53 generally functions as a tumor suppressor. Normally present at low levels in the cells, TP53 expression and activity increase in response to stress to induce cell cycle arrest and DNA damage repair, cellular senescence, or apoptosis.[7] The induction of TP53 activity relies on acetylation of specific lysines, several of which are deacetylated by SIRT1, leading to its inactivation, and suggesting that SIRT1 overexpression would have oncogenic consequences. However, TP53 positively regulates transcription of *SIRT1* and promotes its activity. Therefore, overexpression of SIRT1 would also lead to its own repression via TP53, and direct oncogenic effects of TP53 suppression might not be evident depending on the cellular conditions.

One factor that could impact the outcome of deregulation of such a feedback loop is the cellular localization of the proteins involved. Both SIRT1 and TP53 contain nuclear localization signals and nuclear export signals, allowing for them to shuttle between the nucleus and the cytoplasm under different circumstances.[8,9] In the case of certain cancers, including prostate cancer, lung cancer, breast cancer, and melanoma, SIRT1 has been shown to localize to the cytoplasm, while being located predominantly in the nucleus in the corresponding normal tissues.[10,11] This change in localization could theoretically minimize the deacetylation of TP53 in the nucleus by SIRT1 while still allowing TP53 to regulate its transcription. Thus, in these cancer types under these conditions, the oncogenic role of SIRT1 overexpression through TP53 might be minimized, allowing for other targets of SIRT1 to play a more significant role, especially those that are localized to the cytoplasm. If deacetylation of such targets by SIRT1 were to promote tumor suppression, this could cause SIRT1 to function as a tumor suppressor rather than as a tumor promoter. Cellular localization of SIRT1 also has been shown to differ among different tissue types in mice,[9] which could explain why SIRT1 sometimes exhibits tumor suppressor properties in certain types of cancer but not in others.

A second factor that could impact the role of SIRT1 as a tumor suppressor or tumor promoter is the mutation of proteins with which it would normally interact. For instance, a study comparing *Sirt1*$^{+/-}$, *Trp53*$^{+/-}$, and *Sirt1*$^{+/-}$*Trp53*$^{+/-}$ mice showed that the combined *Sirt1*$^{+/-}$*Trp53*$^{+/-}$ genotype resulted in significantly more tumors by 5 months of age than for either *Sirt1*$^{+/-}$ or *Trp53*$^{+/-}$ alone.[12] Thus, SIRT1 likely plays a tumor suppressor role when TP53 is not present in its wild-type form. Oncogenic activity of SIRT1 can similarly be demonstrated in mice with secondary mutations. A study which crossed a SIRT1 overexpression mouse line with a *Pten*$^{+/-}$ line showed that overexpression of SIRT1 resulted in a significant increase in prostate carcinomas over *Pten*$^{+/-}$ mice aged 5 to 7 months.[13] Thus, SIRT1 plays an oncogenic role in a background of PTEN deficiency. This could be particularly significant considering that PTEN is a suppressor of the phosphoinositide 3-kinase pathway. The previously mentioned study by Byles et al[10] which discovered aberrant cytoplasmic localization of SIRT1 in prostate cancer cells and tissue also showed that inhibition of phosphoinositide 3-kinase reduced the levels of cytoplasmic SIRT1. Taken together, this suggests that the status of both PTEN and TP53 could play a role in determining whether SIRT1 performs as an oncogene or tumor suppressor. Considering the vast number of upstream and downstream proteins with which SIRT1 interacts, it would not be surprising if there were other mutations that would have similar effects.

SIRT1, Aging, and Cancer

It is interesting to note that most of the murine experiments by Di Sante et al[5] were performed in 7-month-old mice. When 3-month-old mice were tested for mitophagy markers, the findings were reversed, indicating that the role of SIRT1 as a tumor suppressor could be age-dependent. This is not surprising considering that SIRT1 has been shown to play a role in aging through its impact on cellular metabolism.[14] The possibility that SIRT1 plays an oncogenic role in younger mice and a tumor suppressor role in older mice could be an important finding for the prevention and treatment of age-related diseases such as prostate cancer.

However, a contradictory view supporting the role of *Sirt1* as an oncogene in aging mice was previously reported by Chen et al[15] and more recently summarized by Deng.[16] Briefly, hypermethylated in cancer-1 (HIC1) is a natural suppressor of SIRT1 transcription in the cell. SIRT1's deacetylation target TP53 transactivates HIC1, forming a regulatory feedback loop among the three proteins. As cells

age, *Hic1* becomes silenced through hypermethylation resulting in the up-regulation of SIRT1 in cancer cells. Again, the overexpression of SIRT1 in cancer cells in aging mice does not necessarily mean that this overexpression is responsible for the cancer formation but likely also depends on the status of proteins which interact with SIRT1. If *Hic1* were to be silenced in tissue with a PTEN deficiency for example, the resultant overexpression of SIRT1 could induce oncogenic events. Thus, the tumor suppressive effects of SIRT1 in aging mice could also be dependent on other factors and not necessarily the case in all genetic backgrounds.

Conclusion

The article by Di Sante et al[5] provides substantial evidence regarding the role of SIRT1 as a tumor suppressor. However, contradictory findings in the literature that point to SIRT1 acting as an oncogene cannot be ignored. It is possible that both scenarios are true depending on a number of factors, including differences in tissue type, species, age, SIRT1 cellular localization, and additional mutations present in conjunction with SIRT1 aberrant expression. In prostate cancer, adequate treatments designed around the molecular target SIRT1 will need to consider a variety of factors, possibly including detailed genetic profiling of specific tumors and concomitant administration of compounds targeting more than one protein with which SIRT1 interacts. The work published here by Di Sante et al[5] will be a valuable contribution toward the development of such therapies in the future.

References

1. Singh BN, Zhang G, Hwa YL, Li J, Dowdy SC, Jiang SW: Nonhistone protein acetylation as cancer therapy targets. Expert Rev Anticancer Ther 2010, 10:935−954
2. Haigis MC, Sinclair DA: Mammalian sirtuins: biological insights and disease relevance. Annu Rev Pathol 2010, 5:253−295
3. Stunkel W, Campbell RM: Sirtuin 1 (SIRT1): the misunderstood HDAC. J Biomol Screen 2011, 16:1153−1169
4. Yuan H, Su L, Chen WY: The emerging and diverse roles of sirtuins in cancer: a clinical perspective. Onco Targets Ther 2013, 6: 1399−1416
5. Di Sante G, Pestell TG, Casimiro MC, Bisetto S, Powell MJ, Lisanti MP, Cordon-Cardo C, Castillo-Martin M, Bonal DM, Debattisti V, Chen K, Wang L, He X, McBurney MW, Pestell RG: Loss of SIRT1 Promotes Prostatic Intraepithelial Neoplasia, Reduces Mitophagy and Delays PARK2 Translocation to Mitochondria. Am J Pathol 2015, 185:266−279
6. Long Q, Xu J, Osunkoya AO, Sannigrahi S, Johnson BA, Zhou W, Gillespie T, Park JY, Nam RK, Sugar L, Stanimirovic A, Seth AK, Petros JA, Moreno CS: Global transcriptome analysis of formalin-fixed prostate cancer specimens identifies biomarkers of disease recurrence. Cancer Res 2014, 74:3228−3237
7. Beckerman R, Prives C: Transcriptional regulation by p53. Cold Spring Harb Perspect Biol 2010, 2:a000935
8. O'Brate A, Giannakakou P: The importance of p53 location: nuclear or cytoplasmic zip code? Drug Resist Updat 2003, 6:313−322
9. Tanno M, Sakamoto J, Miura T, Shimamoto K, Horio Y: Nucleocytoplasmic shuttling of the NAD+-dependent histone deacetylase SIRT1. J Biol Chem 2007, 282:6823−6832
10. Byles V, Chmilewski LK, Wang J, Zhu L, Forman LW, Faller DV, Dai Y: Aberrant cytoplasm localization and protein stability of SIRT1 is regulated by PI3K/IGF-1R signaling in human cancer cells. Int J Biol Sci 2010, 6:599−612
11. Wilking MJ, Singh C, Nihal M, Zhong W, Ahmad N: SIRT1 deacetylase is overexpressed in human melanoma and its small molecule inhibition imparts anti-proliferative response via p53 activation. Arch Biochem Biophys 2014, 563:94−100
12. Wang RH, Sengupta K, Li C, Kim HS, Cao L, Xiao C, Kim S, Xu X, Zheng Y, Chilton B, Jia R, Zheng ZM, Appella E, Wang XW, Ried T, Deng CX: Impaired DNA damage response, genome instability, and tumorigenesis in SIRT1 mutant mice. Cancer Cell 2008, 14:312−323
13. Herranz D, Maraver A, Canamero M, Gomez-Lopez G, Inglada-Perez L, Robledo M, Castelblanco E, Matias-Guiu X, Serrano M: SIRT1 promotes thyroid carcinogenesis driven by PTEN deficiency. Oncogene 2013, 32:4052−4056
14. Canto C, Auwerx J: Caloric restriction, SIRT1 and longevity. Trends Endocrinol Metab 2009, 20:325−331
15. Chen WY, Wang DH, Yen RC, Luo J, Gu W, Baylin SB: Tumor suppressor HIC1 directly regulates SIRT1 to modulate p53-dependent DNA-damage responses. Cell 2005, 123:437−448
16. Deng CX: SIRT1, is it a tumor promoter or tumor suppressor? Int J Biol Sci 2009, 5:147−152