The role of SIRT6 in tumors

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Dysfunctional DNA-damage response and consequent genomic instability play a pivotal role in the initiation and progression of both solid and hematologic tumors. Preservation of DNA integrity is, in fact, a key cellular function, hence several mechanisms that repair the damaged DNA need to be studied. Recent studies have focused on key players that are able to improve the DNA repair and thus may act as targets for new therapeutic approaches.

Several data have been obtained on overexpression and hyperactivity of Sirtuins (SIRTs), a family of proteins with deacylase or mono-adenosine diphosphate (ADP)-ribosyltransferase activities that degrade nicotinamide adenine dinucleotide (NAD^+) enzymes to enable their biological processes[1] and promote longevity.[2] In mammalian cells, the Sirtuin family is composed of seven members that show different subcellular localization and functions (transcription, metabolism, fat mobilization, DNA repair, stress responses, apoptosis, tumorigenesis and aging),[3,4] and conserve the catalytic domain and the NAD^+ binding site.[5] In cancer and aging-associated pathways, SIRT6 is crucial since it prevents genomic instability, maintains telomere integrity, and regulates metabolic homeostasis and DNA repair.[6] SIRT6 can be considered a double-edged sword in cancer because of its dual role of both tumor suppressor and oncogene (Table 1). In healthy conditions, SIRT6 either acts as a gatekeeper of DNA repair mechanisms or regulates cell survival and proliferation. Following the DNA damage, SIRT6 triggers the apoptotic process, hence it is down-regulated in several cancers. However, in other cancers, it is up-regulated, corroborating the idea that it can also act as oncogene.

SIRT6 as a tumor suppressor

Studies in colorectal, breast, ovarian, hepatocellular, lung, and other tumors correlate the reduction of SIRT6 expression with tumor progression and poor clinical outcome. In the presence of DNA-damage, SIRT6 promotes apoptotic cell death, ensuring damaged cells do not proliferate. Sebastian *et al.*[7] demonstrated *in vivo* that SIRT6 deficiency favors tumor growth and invasiveness. They also showed that SIRT6 is involved in the Warburg effect, a glycolytic metabolic shift important for supporting rapid tumor growth. SIRT6 promotes both *in vitro* and *in vivo* tumor suppression through repression of hypoxia-inducible factor 1-alpha (HIF-1α) that inhibits glycolytic metabolism in cancer cells.[7] Interestingly, in mouse and human pancreatic ductal adenocarcinoma (PDAC), the SIRT6 knockdown is due to repression of Myc-target oncofetal protein Lin28b that negatively regulates the let-7 family of miRNAs.[8] In detail, loss of SIRT6 triggers activation of Lin28 promoter, Myc recruitment, and consequent activation of Lin28b, the downstream let-7 target genes (*HMGA2*, *IGF2BP1*) and IGF2BP3 that accelerate the PDAC progression and metastasis.[8] In human colon cancer, Lin *et al.*[9] discovered the crosstalk between UPS10 and SIRT6 that regulates cell-cycle progression and proliferation, and showed that the dysregulated USP10 function promotes tumorigenesis through SIRT6 degradation. Lin *et al.* also showed an important reduction in USP10 (a deubiquitinase protein) and SIRT6 expression. Indeed, the downregulation of USP10 triggers SIRT6 instability and negatively controls the transcriptional activity of the c-Myc oncogene that inhibits cell-cycle progression, cancer cell growth, and tumor formation.[9] In liver cancer, the SIRT6 suppression is regulated by the c-Jun/c-Fos pathway:[10] c-Fos induces SIRT6 transcription and represses survivin by reducing histone H3K9 acetylation and NF-κB activation. The increase in SIRT6 impairs cancer development by targeting the anti-apoptotic activity of survivin. Min *et al.*[10] identified in human dysplastic liver nodules a specific expression pattern characterized by increased c-Jun-survivin and reduced c-Fos-SIRT6 level. In hepatocellular carcinoma (HCC), Bhardwaj *et al.*[11] found that SIRT6 acts as a tumor suppressor because it deacetylates nuclear pyruvate kinase M2 (PKM2) inhibiting cell proliferation and tumorigenesis *via* PKM2. In ovarian cancer, Zhang *et al.*[12] showed that SIRT6 is down-regulated at mRNA and protein levels in tumor cells compared to normal cells. Moreover, SIRT6 reduces the expression of neurogenic locus notch homolog protein 3 (Notch3) while the Notch3 overexpression antagonists SIRT6 exert an effect on the ovarian cell proliferation; SIRT6 thus inhibits the proliferation of ovarian tumor cells through regulation of Notch3.[12] In breast cancer, the repression of SIRT6, mediated by runt-related transcription factor 2 (RUNX2), regulates metabolic pathways and promotes tumor development.[13] More specifically, Choe *et al.*[13] showed that RUNX2 down-regulates the SIRT6 expression at both mRNA and protein levels, and that endogenous SIRT6 expression is lower in the tumor breast tissue and cell lines expressing high levels of RUNX2 regulating the metabolic pathways. In addition, Han *et al.*[14] demonstrated in non-small cell lung cancer (NSCLC) that SIRT6 inhibits Twist1 expression. Twist1 is a member of basic helix-loop-helix transcription factor family that promotes tumor proliferation and malignant transformation. Thus, SIRT6 is able to inhibit tumor cell proliferation through Twist1 suppression. Finally, the overexpression of E2F transcription factor 1 (E2F-1) in bladder and prostate cancer induces the downregulation of SIRT6 that closely correlates with cancer progression and poor prognosis.[15]

SIRT6 as a tumor promoter

In contrast to these studies, several papers show that overexpression of SIRT6 in solid and in hematologic tumors can promote oncogenic activity. Ming *et al.*[16] demonstrated the oncogenic role of up-regulated SIRT6 in human skin squamous cell carcinoma (SCC): in skin keratinocytes, SIRT6 is

increased upon exposure to ultraviolet B (UVB) light through the activation of the AKT pathway and promotes the cyclooxygenase 2 (COX-2) expression that represses the AMP-activated protein kinase (AMPK) signaling and increases proliferation and cell survival.[16] Zhang *et al.*[17] demonstrated that SIRT6 overexpression in HCC suppresses tumor growth by blocking extracellular signal-regulated kinases (ERK) 1/2 signaling pathway. In addition, Feng *et al.*[18] and Ran *et al.*[19] showed that SIRT6 plays an oncogenic role in HCC. In particular, the overexpression of SIRT6 is required for induction of transforming growth factor (TGF)-β1 and H2O2/HOCl reactive oxygen species (ROS) that mediate tumorigenesis. TGF-β1 up-regulates the SIRT6 expression inducing the activation of ERK and Smad pathways, and altering the effect of these proteins on cellular senescence.[18] Ran *et al.*[19] demonstrated an oncogenic effect of SIRT6 *via* chromatin remodeling. At molecular level, SIRT6 induces deacetylation of H3K9 that blocks Bcl-2-associated X protein (Bax) transcription. As a consequence, it enhances p53 and E2F-1 chromatin accessibility thus inhibiting apoptosis. Elhanati *et al.*[20] and Lefor *et al.*[21] correlated SIRT6 regulation to two microRNAs (miR-) in two different cancers. At basal conditions, SIRT6 and miR-122 negatively regulate each other in HCC. SIRT6 down-regulates miR-122 by deacetylating H3K56 in the promoter region. The miR-122 binds SIRT6 3′ UTR and reduces its levels, while the loss of the negative correlation between SIRT6 and miR-122 expression is significantly associated with better prognosis.[20] In addition, miR-34a plays a key role during

Table 1. SIRT6 expression and its role in cancer.

SIRT6 status	Study	Cancer type	Role of SIRT6	Pathways and regulators
↓	Sebastian[7]	Colorectal	Warburg effect and tumor growth	Suppression of Myc-regulated genes (HIF-1)
↓	Min[10]	Liver	Tumorigenic effect	Increased levels of c-Jun-survivin and reduced levels of c-Fos-SIRT6
↓	Lin[9]	Colon	Cancer cell growth; tumorigenic effect	Reduction of deubiquitinase protein USP10 antagonizes the transcriptional activity of the c-Myc oncogene
↓	Han[14]	Non-small cell lung	Inhibition of tumor proliferation and malignant transformation	Inhibition of Twist1 expression
↓	Choe[13]	Breast	Reduction of mitochondrial oxygen consumption rates or respiration	Regulation of RUNX2-mediated metabolic changes increases pAkt, HK2, and PDHK1 glycolytic protein level
↓	Wu[15]	Bladder and prostate	E2F1b suppresses SIRT6 transcription	E2F1 binds to SIRT6 promoter and suppresses its activity
↓	Zhang[12]	Ovarian	Inhibition of proliferation	Reduction of Notch 3 expression
↓	Bhardwaj[11]	Hepatocellular carcinoma	Inhibition of cell proliferation and tumorigenesis *via* nuclear pyruvate kinase M2 (PKM2)	Deacetylation of PKM2
↓	Kugel[8]	Pancreatic ductal adenocarcinomas	Loss of SIRT6 accelerates tumor progression and metastasis	Activation of Lin28 and the downstream let-7 target genes (*HMGA2*, *IGF2BP1*, and *IGF2BP3*)
↑	Lefort[21]	Squamous cell carcinoma	miR-34a target	Expression of miR-34a induces cell differentiation through DNA methylation or p53
↑	Ming[16]	Squamous cell carcinoma	Proliferation and tumor cell survival by means of UVB	Activation of AKT pathway and COX-2 expression repressing AMPK signaling
↑	Zhang[17]	Hepatocellular carcinoma	Tumor growth suppression	ERK1/2 signaling pathway
↑	Feng[18]	Hepatocellular carcinoma	Tumorigenesis	TGF-β1/H2O2/HOCl ROS up-regulate SIRT6 expression by ERK and Smad pathways
↑	Elhanati[20]	Hepatocellular carcinoma	miR-122 target and poor prognosis	Deregulated miR-122 binds to three sites on the SIRT6 3' UTR and reduces its level
↑	Ran[19]	Hepatocellular carcinoma	Block of apoptosis	Deacetylation of H3K9 blocking Bax transcription and enhancing p53 and E2F-1 chromatin accessibility
↑	Cea[22]	Multiple myeloma	Genome instability and poor prognosis	Increased ELK1 and ERK signaling-related gene
↑	Cagnetta[24]	Acute myeloid leukemia	Genome instability and poor prognosis	In $CD34^+$ blasts, SIRT6 expression is associated to ongoing DNA damage and intense replicative stress

UVB: ultraviolet B cancer.

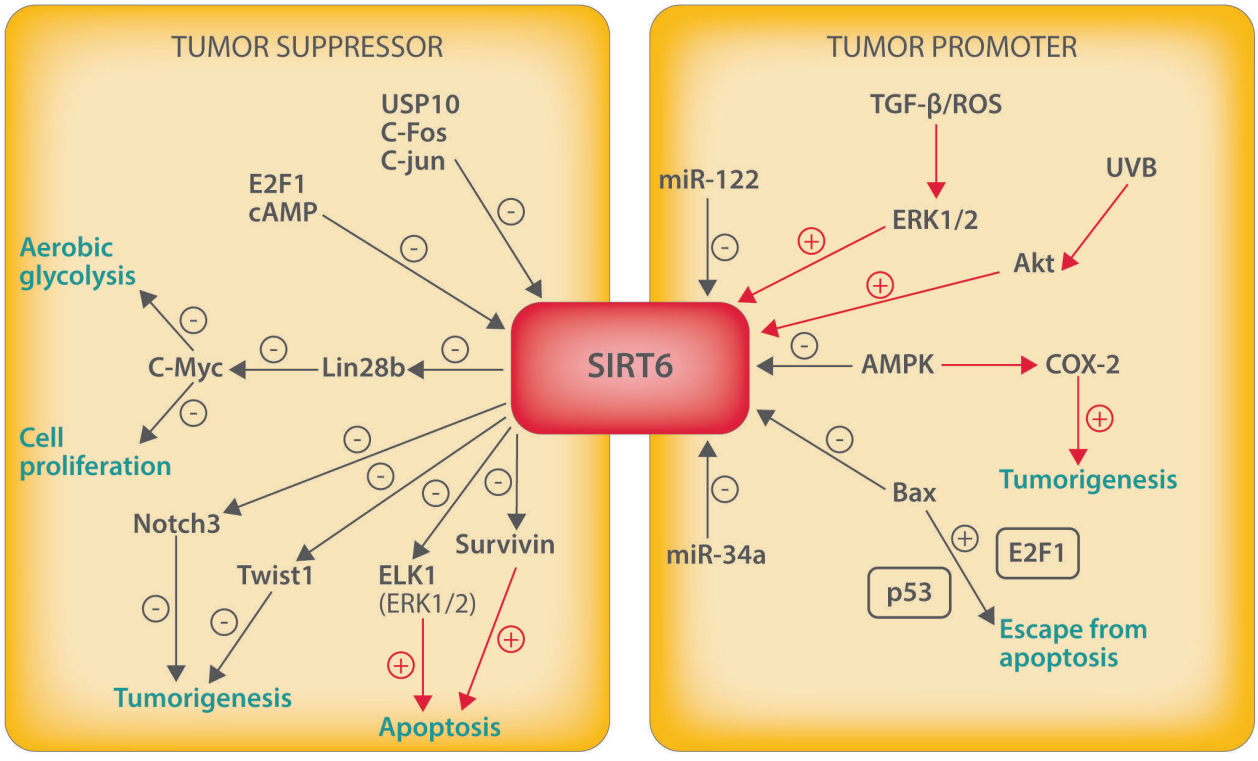

Figure 1. SIRT6 in cancer acts as tumor suppressor and tumor promoter in different cellular pathways.

the differentiation process of HCC and SIRT6 represents one of its targets. SIRT6 downregulation induces differentiation effects mediated by miR-34a.[21]

The role of SIRT6 is not well known in hematologic malignancies. In multiple myeloma (MM), SIRT6 is highly expressed as adaptive response to genomic stability, and its overexpression is associated to proliferation and poor prognosis.[22] Cea *et al.*[22] demonstrated *in vitro* and in a human MM xenograft model that SIRT6 down-regulates the expression of ERK signaling-related genes and suppresses the activity of ETS-domain transcription factor (ELK1), increasing DNA repair level *via* Chk1 (a critical messenger of the genome integrity checkpoints involved in the evolution of human cancer[23]), and conferring resistance to DNA-damaging agents. In this scenario, the paper by Cagnetta *et al.*[24] studies the biological relevance and the genomic instability and poor prognosis associated with the mRNA upregulation of SIRT6 in the acute myeloid leukemia (AML) cells compared with low SIRT6 levels detected in normal CD34$^+$ hematopoietic progenitors. SIRT6 participates in DNA double-strand break repair by deacetylation of C-terminal binding protein (CtBP), interacting protein (CtIP), poli ADP-ribosio polimerase-1 (PARP-1) and DNA-protein kinase (PK) complex. Indeed, AML cells are able to recruit SIRT6 in DNA-damaged sites and to promote deacetylation by means of DNA-PKs and CtIP. On the contrary, downregulation of SIRT6 expression both *in vitro* and in a murine xenograft model of human AML promotes genomic instability that sensitizes AML cells to daunorubicin (DNR) and cytarabine (ARA-C). Importantly, the results from Cagnetta *et al.* suggest an innovative chemotherapy that may selectively target AML cells enhancing their sensitivity to DNA-damage agents (DDAs).[24]

In conclusion, SIRT6 fulfills a controversial role in the pathogenesis of several cancers (Figure 1). It is clear that SIRT6 plays a crucial role in the regulation of tumorigenesis through its implication in different biological pathways where it can act as tumor suppressor or oncogene. The pleiotropism of SIRT6 means that studies directed toward understanding the cellular mechanisms through which the Sirtuin impacts cancer are difficult to carry forward but tremendously exciting. As Cagnetta *et al.* suggest,[24] it is important that SIRT6 be included in the prospective clinical trials as a novel strategy of anti-tumor therapy.

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Remission is good - relapse is bad

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The prognostic significance of minimal residual disease (MRD), or perhaps 'measurable' residual disease,[1] is well-established acute and chronic leukemia.[2,3] The vast effort of European investigators in standardizing MRD assessment by polymerase chain reaction (PCR) and flow cytometry merits recognition and credit.[4,5] At present, we have several independent quantitative monitoring strategies, namely, PCR on DNA targets, reverse transcription (RT)-PCR on abnormal ribonucleic acid (RNA) transcribed from fusion genes or overexpression of normal messenger (m)RNA, and flow cytometry. Their relative implications remain under investigation.

MRD results, whatever the target, depend on specimen quality. Marrow aspirates represent a variable mixture of marrow and peripheral blood. Sensitivity depends on the number of cells or amount of nucleic acid interrogated. Leukemia may present with uniform marrow replacement and remit homogeneously across the marrow. Early relapse, however, may be patchy or perhaps anatomically localized with only later dissemination. Peripheral blood may be of use, despite a consistently lower and not always predictable presence of leukemic blasts in the peripheral blood relative to the bone marrow.[6]

The comparison of quantitative MRD strategies based on DNA and RNA is complex. The DNA target may persist from residual dying cells or in cells lacking leukemogenic potential, vis-à-vis the persistence of *DNMT3A* mutations in acute myeloid leukemia (AML),[7] representing clonal hematopoiesis and not always associated with relapse. While one or two copies of DNA targets are present per cell, the expression of both the target RNA and the housekeeping genes employed as denominators can vary from patient to patient, and from cell to cell for individual patients. Interventions may affect gene expression as well as cell number. The RNA target may also be present in cells lacking leukemogenic potential. RNA is more labile than DNA.

In this issue of Haematologica, Cazzaniga *et al.* compare MRD monitoring by RQ-PCR of DNA-based rearranged immunoglobulin/ T-cell receptor gene rearrangements (IG/TR), and of RNA-based *BCR/ABL1* fusion transcript in 90 young people with Philadelphia chromosome-positive acute lymphoblastic leukemia (PH+ ALL) who were allocated to imatinib on the European intergroup study of post-induction treatment of PH+ ALL (EsPhALL; EudraCT 2004-0014647-30; *clinicaltrials.gov Identifier: 00287105*). Of the 57 patients characterized, about 90% had the p190 transcript and 10% the p210 transcript.[8] Imatinib treatment was initiated after the first time point (tp1), at the completion of Induction IA at 5-7 weeks from diagnosis, and continued intermittently. Contemporary protocols for PH+ ALL begin tyrosine kinase inhibitors earlier and continue them without interruption.

None of the nine patients with undetectable MRD by PCR targeting IG /TR after one month of therapy (end induction IA) relapsed. MRD positive patients had a similar ~35% relapse rate, whether MRD was quantifiable (≥