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Editorial

Mutational and Epimutational Analysis of Cell Death-Resistant Tumor Cells: Clues to Molecular Carcinogenesis and Cancer Therapy

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Editorial

Cancer is a versatile complex disease and the development of a wide arsenal of treatment options is based upon piles of accumulated basic molecular research. This basis increasingly enhanced our knowledge of fundamental mechanisms underlying tumorigenesis [1]. Besides studies involving *in-vivo* studies using patient´s material and xenografted mice, the performance of *in-vitro* experiments using tumor cells has led to the accumulation of a huge amount of data and significant progress. Among these insights was the revelation of the development of oncogenic transformation at several cellular levels due to silenced tumor suppressor genes, gain-of-function of oncogenes, loss-of cell death resistance, dysregulated intracellular signaling pathways, epithelial to mesenchymal transitions and dysregulated metabolism.

Genetic irregularities have been considered as distinctive features of a tumor cell since a long time [2]. Analysis of tumor-specific mutations in human genomic DNA was initially demonstrated by sequencing the Kras gene [3]. For the generation of *in-vitro* insights obtained of tumor biology and genetics since then, tumor-derived cell lines have taken over an important role. Across a wide array of studies, they have been tools of choice for gene and cellular pathway analysis that are impaired by diverse oncogenic events. They are also exceptionally indispensable for the screening of novel anticancer drugs.

In recent times, furthermore, epigenetic studies gained increasing significance in reports investigating the development of cancer [4]. For this purpose, deviant epigenome including the misguided expression of HDACs activity has been defined to some extent in diverse tumor entities [5]. As a result, it was concluded that aberrant epigenetic patterns such as DNA methylation and histone modifications are very common in tumor cells. Histone modifications, predominantly acetylation or deacetylation, are executed by different enzyme classes of histone acetyltransferases (HAT) and histone de-acetylases (HDAC), respectively [6]. Nevertheless, HDAC not only catalyze the removal of the acetyl groups from histones, but also from non-histone proteins and enable pharmacological interference by different kind of inhibitors. The physiological functions of HDAC are still not fully elucidated but involve key cellular processes such as transcriptional regulation, apoptosis, cell cycle control, and autophagy that are involved in tumorigenesis. Conclusively, cancer is furthermore not only regarded as a pathologic condition of altered genetic, but also of epigenetic, deregulation.

Several large tumor genome sequencing projects are ongoing or have been completed in recent years (e.g. the Cancer Genome Project by the Wellcome Trust Sanger Institute) to get an overview of entire genome variations that occur in a tumor cell and to elucidate novel cancer genes and potential therapeutic targets [7]. An unpredicted discovery resulting from these projects was that along with the small number of original cancer-causing "driver mutations" that enforce tumor progression, numerous so-called "passenger mutations", which seem to be mostly insignificant for tumorigenesis, are co-selected and carried by the tumor cell [8]. Nevertheless, as the role these passenger mutations is currently unexplored they should not be underrated with regard to potential future findings and and it has been proposed that driver mutations engage in a tug-of-war with damaging passengers [9,10].

Apoptotic cell death is one of the key mechanisms frequently inactivated in cancer cells and might be attributed to driver mutations during cancer progression [11,12]. Defects in type I (apoptosis) programmed cell death facilitate tumor development and diminish chemotherapy, suggesting that an escape route to another pathway such as cell death type II (autophagy) or type III (necrosis) may be favored and therapeutically beneficial. Therefore, identification of the affected molecules with subsequent screening for agents that abolish cell death resistance and re-activate apoptosis might clear the way to functional anti-cancer therapeutics. By disclosing a genomic as well as epimutations in two different uterine sarcoma cell lines, we were recently able to unravel diverse resistance mechanisms of apoptosis and autophagy in this context.

In the first study, we searched for the cause of varying cellular responses elicited by two human uterine sarcoma cell lines upon treating them with the histone deacetylase inhibitor (HDACi) SAHA [13,14]. While, pronounced activation of apoptosis was determined in MES-SA uterine sarcoma cells in xenografts as well as *in vitro*, predominant SAHA-mediated dose-dependent autophagic cell death was observed in ESS-1 cells followed by inactivation of mammalian target of rapamycin (mTOR) which is a known regulator of autophagy [15]. In our quest for responsible upstream autophagic regulatory genes, we found that expression of the tumor suppressor protein p53 was entirely lacking in ESS-1 cells when compared to the easily detectable and abundant expression in MES-SA cells. As a cause for this difference, a nonsense mutation (TP53-637C>T) located in the

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trans-activating domain of the oncogenic suppressor p53 could be identified which causes loss of protein expression and consequently reduced PUMA induction in ESS-1 cells. As a proof of concept, the restoration of SAHA-treated ESS-1 cells by functional p53 expression provoked immediate cell death through apoptosis as evidenced by PUMA upregulation, caspase activation, and PARP-1 cleavage. Concurrent downregulation of autophagy to physiologic basal levels was recorded by re-elevated mTOR expression and by monitoring autophagosome formation. General validation of an inhibitory role for p53 in the autophagic pathway was obtained by RNAi-silenced p53 expression in MES-SA uterine sarcoma cells and by additional p53-deficient tumor cells that underwent SAHA-induced autophagy. Thus, we reasoned that the oncogenic suppressor p53 might act in addition to its many other regulatory functions as a molecular switch that directly drives the cytotoxic response of SAHA towards apoptosis or autophagy. These findings would be in accordance with a previous set of studies indicating that the p53 protein has a master regulatory activity by being able to promote as well as inhibit the autophagic process, depending on its subcellular localization. Consistently, p53-deficiency could account for the previously documented apoptosis resistance and prevailing SAHA-induced autophagy in ESS-1 cells. If such a mechanism can be confirmed *in vivo*, the mutational status of p53 in tumors will be crucial as it will significantly influence the outcome of therapeutic HDACi cancer treatment.

In the second study, the uterine sarcoma cell lines MES-SA and ESS-1 were found to be highly resistant to death receptor-induced apoptosis upon single treatment with tumor necrosis factor-related apoptosis-inducing ligand (TRAIL/Apo-2L) [16]. As in comparison to single TRAIL treatment, the additional combined administration with the histone de-acetylase (HDAC) inhibitor suberoylanilide hydroxamic acid (SAHA) led to accelerated induction of apoptosis. SAHA- and TRAIL-induced apoptosis was accompanied by upregulation of the intrinsic apoptotic pathway as evidenced by a decrease in the mitochondrial membrane potential, caspase activation, and PARP-1 cleavage. Therefore, we decided to investigate the underlying molecular mechanisms of TRAIL resistance. As a direct cause, we detected reduced expression of caspase-8 and DR 4/TRAIL-R1 in ESS-1 and MES-SA cells, respectively. As the originating cause we detected epigenetic silencing of gene promoter sequences due to DNA hypermethylation. This clearly explained how TRAIL generated signals were not at all, or less efficiently transduced, and how these tumor cells escaped death receptor-induced apoptosis. DNA demethylation by 5-Aza-2'-deoxycytidine or genetic rescue was found to restore gene expression and increase the sensitivity of both cell lines against TRAIL-induced apoptosis.

Taken together, these insights from both studies underline that epigenetic deregulation either by histone acetylation or by DNA hypermethylation might provide the basis for the development of uterine sarcomas and allow advanced therapeutic options based on interference with epigenetic regulatory mechanisms. Thus, the identification of a mutation and epimutations in uterine sarcoma cells have revealed apoptosis escaping routes and might have pointed to an unknown regulatory mechanism of SAHA-induced autophagy. Recent progress in sequencing methods i.e. next-generation sequencing has already allowed the description of numerous somatic and epigenetic changes in the whole genome tumor cells. A key challenge will however consist in differentiating which of the alterations of cancer genomes and epigenomes are "drivers" or "passengers" of cancer development in which our functional approaches might be useful.

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