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Lysosomotropic REV-ERB antagonism: A metabolic connection between circadian rhythm and autophagy may tell cancer cells "it's time to die"

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The discovery that inhibition of a circadian regulator enhances autophagy-dependent cancer cell death reveals potential avenues for the development of new multifunctional anticancer agents. Further studies may elucidate novel crosstalk between circadian rhythm, metabolism, and autophagy that determines cancer cell viability.

Autophagy is a self-degradation process by which cells consume parts of themselves to survive starvation and stress.[1,2] In normal cells, autophagy has emerged as a useful defense system: it limits cell death and tissue inflammation, recycles energy and biosynthetic substrates, and prevents the accumulation of damaged proteins and organelles, which is otherwise toxic. In cancer cells, the role of autophagy is complex: it may either inhibit or promote tumor growth, depending on the cellular context.[3]

Autophagy may suppress tumor growth in the tumor initiation stage, whereas in established tumors it may fulfill the metabolic needs of cancer cells during oncogene activation or nutrient limitation by providing a mechanism to recycle intracellular carbon and nitrogen. The efficacy of autophagy inhibition in cancer therapy is well documented in preclinical models, and the lysosomotropic autophagy inhibitor chloroquine (CQ) and its analog hydroxychloroquine (HCQ) are currently being evaluated in numerous cancer clinical trials.[4]

We recently reported that the reverse of ERBα-β variant protein (REV-ERBβ), a nuclear receptor involved in circadian rhythm and metabolism, plays an unexpected role as a determinant of sensitivity to chloroquine.[5] Although REV-ERBβ does not seem to contribute to cancer cell viability *per se*, genetic inhibition of this nuclear receptor sensitized breast cancer cells to chloroquine-induced cytotoxicity.

Studies in mice revealed that REV-ERBβ and its variant, the reverse of ERBα protein (REV-ERBα), compensate for one another in the repression of common target genes.[6] Surprisingly, our study showed that depletion of REV-ERBα by RNA interference had negligible effects on chloroquine-mediated cell death. REV-ERBβ–specific sensitization to chloroquine is likely due to the marked upregulation of this variant in cancer cells. In fact, in marked contrast to normal epithelial cells, we found that REV-ERBβ is the predominantly expressed REV-ERB variant in a number of cancer cell lines. Knockdown experiments also indicated that in cancer cells REV-ERBβ is the principal transcriptional regulator of circadian and metabolic REV-ERB targets such as the clock master regulator, brain and muscle arnt-like protein-1 (BMAL1), and the gluconeogenetic phosphoenolpyruvate carboxykinase enzyme PEPCK.

Consistent with the expression pattern in cancer cell lines, we observed that although REV-ERBα was more abundant than REV-ERBβ in various normal human tissues, the latter was more highly expressed in several tumor samples of different origin.

The fact that a crucial regulator of circadian rhythm and metabolism affects sensitivity to autophagy inhibition in cancer cells has several therapeutic implications.

The efficacy and the collateral toxicity of different many drugs, including antitumor agents, are greatly affected by circadian timing and recent studies have revealed that adopting an anticancer chronotherapeutic strategy leads to better therapeutic outcomes.[7] Because REV-ERBβ expression follows a circadian oscillatory pattern, chronopharmacokinetic studies of autophagy inhibitors may improve their antitumor efficacy.

In addition, combinatory inhibition of both REV-ERBβ and autophagy may offer a novel pharmacological approach to induce cytotoxicity in cancer cells. Following this idea, we identified a novel REV-ERBβ antagonist, ARN5187, which also possesses lysosomotropic properties. Accordingly, ARN5187 is a novel autophagy inhibitor that disrupts lysosomal function and blocks the latter stages of autophagy. In addition, ARN5187

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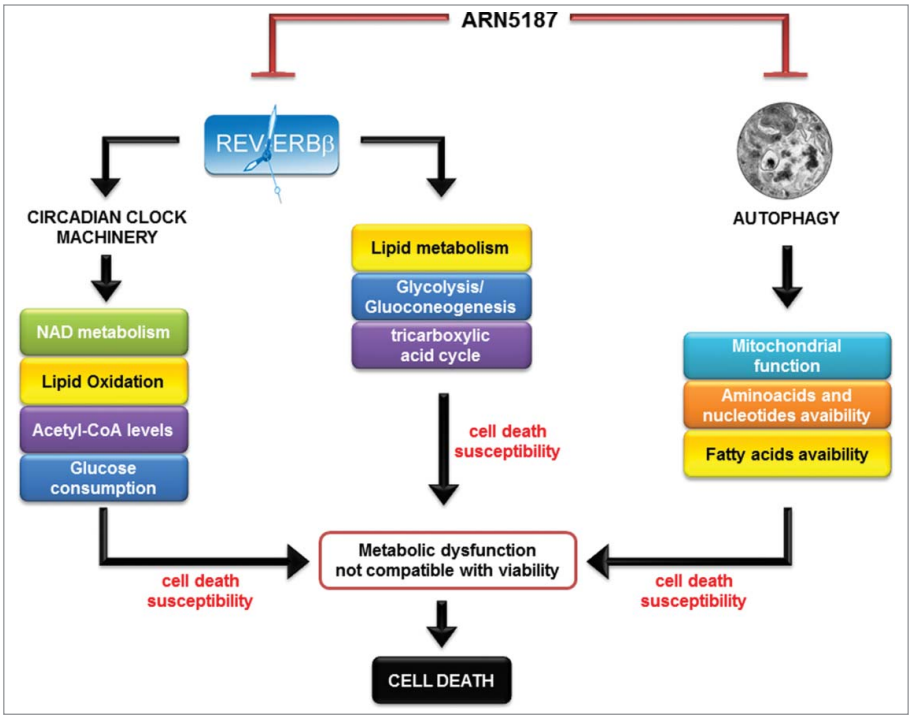

Figure 1. Mechanism of action of ARN5187. According to this tentative model, inhibition of reverse of ERBα-β variant protein, REV-ERBβ, and inhibition of autophagy cooperate to induce a metabolic dysfunction that is incompatible with cancer cell viability. REV-ERBβ antagonism will affect metabolism at different levels, regulating the expression of key circadian and metabolic genes. The resulting compromise in metabolic pathways renders cancer cells susceptible to a lethal "second hit" that is derived from the blockade of autophagy.

inhibits REV-ERBβ activity and relieves REV-ERB-mediated transcriptional repression.

Although ARN5187 and chloroquine have similar lysosomotropic potency and are equivocal with regard to autophagy inhibition, ARN5187 is significantly more cytotoxic. This is consistent with the notion that REV-ERB–dependent activity exerts a cytoprotective function when the autophagy flux of cancer cells is compromised. Although further *in vivo* studies are needed to fully evaluate the potential of ARN5187 or its analogs as anticancer agents, our data suggest that multifunctional inhibition of REV-ERBβ and autophagy may provide a novel approach for the development of new anticancer drugs.

Furthermore, the ARN5187 series is a valuable pharmacological tool for in-depth analysis of the role of REV-ERBβ and autophagy in the induction of cancer cell death. Our data indicate that REV-ERBβ does not seem to be directly involved in autophagy regulation, but likely acts as a cytoprotective factor downstream of a blockade of autophagy. Considering the number of inter-connected responses that are regulated by REV-ERB proteins (i.e., circadian rhythm, metabolism, and inflammation),[8,9] the molecular mechanism(s) underlying this prosurvival function may involve different pathways. Nevertheless, the crucial position of REV-ERB proteins in the regulation of cellular metabolism suggests a provocative hypothesis in which the inhibition of both REV-ERBβ and autophagy cooperate to induce a metabolic dysfunction that is incompatible with cancer cell viability. The variable sensitivity of cells to induction of autophagy and cell death is due to the status of both the extra- and intracellular metabolic environments.[10] Accordingly, autophagy can play a prosurvival role in established cancer because of the high energy demand of cancer cell metabolism.[3] In mouse liver, genetic ablation of REV-ERB resulted in several metabolic abnormalities; this is consistent with the direct recruitment of REV-ERBβ to the promoters of several genes involved in the regulation of glycolysis/gluconeogenesis, the tricarboxylic acid (TCA) cycle, and lipid metabolism.[6] In addition, because of the intimate link between the circadian clock and cellular metabolism,[8,9] REV-ERB-mediated regulation of the molecular clock machinery further places REV-ERB proteins at a strategic position in metabolism.

These observations support a scenario in which REV-ERBβ inhibition may generate a non-lethal metabolic stress in cancer cells that sensitizes them to a lethal "second hit" when the autophagy flux is blocked (**Fig. 1**). Implicit in this hypothesis is the concept that the altered circadian regulation found in several tumors may be triggered by the special metabolic needs of the cancer cell.

Further studies in this area may reveal novel interconnections between the circadian clock, autophagy, and metabolism that are required for cancer cell survival.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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