

# Tricking an ancient immune function to eradicate hepatocellular carcinoma

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**Abbreviations:** AIM, apoptosis inhibitor of macrophage; DDS, drug delivery system; FASN, fatty acid synthase; HCC, hepatocellular carcinoma; HFD, high-fat diet; RCA, regulator of complement activation.

The circulating apoptosis inhibitor of macrophage (AIM) protein performs differential preventive roles against liver steatosis and hepatocellular carcinoma (HCC) development. Since non-alcoholic fatty liver disease, which is known as a manifestation of metabolic syndrome, has been increasingly highlighted for its association with HCC, therapeutic application of AIM would open the door toward a new mode of treatment for modern hepatic diseases.

Researchers have attempted to overcome the threat of cancer using 2 principal strategies. The first of these is to understand the enigma of carcinogenesis, namely the molecular mechanisms by which cancer cells develop. The second is to target existing cancer cells so that they are eliminated. It goes without saying that a huge number of anticancer compounds have been generated to achieve this second aim and are currently used in a variety of combinations. Many of these compounds do eliminate tumors, or at least slow disease progression, but there are almost always some serious side effects that accompany their use. However, it is worth remembering that humans have survived cancer for more than 1.6 million years, and for most of that period we have lacked specific therapeutic tools. This suggests that we must have our own internal system that distinguishes newly developing cancer cells from the surrounding normal cells in tissue and can eliminate these undesirable cells. If so, this self-guarding system must be very effective, and there is therefore no reason not to apply it to current cancer therapy. In our most recent work, published in *Cell Reports*,<sup>1</sup> we

presented the existence of a protective system based on a circulating protein, apoptosis inhibitor of macrophage (AIM), that is active in hepatocellular carcinoma (HCC) (Fig. 1). HCC is a well-known chemotherapy-resistant tumor and the third most common cause of cancer-related deaths.

AIM, also called CD5-like antigen (CD5L), is a member of the scavenger receptor cysteine-rich superfamily and was initially identified as a supporter of macrophage survival.<sup>2</sup> AIM is produced solely by tissue macrophages, and its transcription is regulated by nuclear receptor liver X receptor/retinoid X receptor heterodimers.<sup>3,4</sup> Interestingly, AIM associates with the IgM pentamer in blood, which protects AIM from renal excretion and thus maintains a high level of circulating protein (approximately 2.5–10 µg/ml).<sup>5</sup> In contrast to many other soluble proteins, AIM does not mediate signal transduction in target cells, but is incorporated via scavenger receptor-mediated endocytosis. Typically, AIM is endocytosed through CD36 into adipocytes and hepatocytes, where it binds to and inactivates cytoplasmic fatty acid synthase (FASN). This

leads to a reduction in lipid droplet-coating proteins, such as fat-specific protein 27 and perilipin, which in turn decreases triacylglycerol deposition within the cells.<sup>1,6,7</sup> Overall, these effects of AIM result in prevention of obesity and fatty liver disease. In AIM-deficient (*AIM*<sup>-/-</sup>) mice fed an high-fat diet (HFD), the rate of visceral adipose tissue mass increases and the degree of liver steatosis is greater than that in wild-type mice fed an HFD; this phenotype is abolished by administering recombinant AIM (rAIM) to *AIM*<sup>-/-</sup> mice.<sup>1,6</sup> Thus, circulating AIM functions as a “brake” against fat deposition in the body.

However, the situation changes in cancerous hepatocytes. Defective endocytosis is a common characteristic of many different types of cancer cell, and results in insufficient incorporation of AIM into HCC cells after its binding to extracellular scavenger receptors. Instead, AIM accumulates on the cell surface.<sup>1</sup> As such, AIM accumulation can be used to distinguish HCC cells from normal background hepatocytes. Furthermore, cell surface AIM specifically stimulates HCC cell death by necrosis, thereby preventing tumor

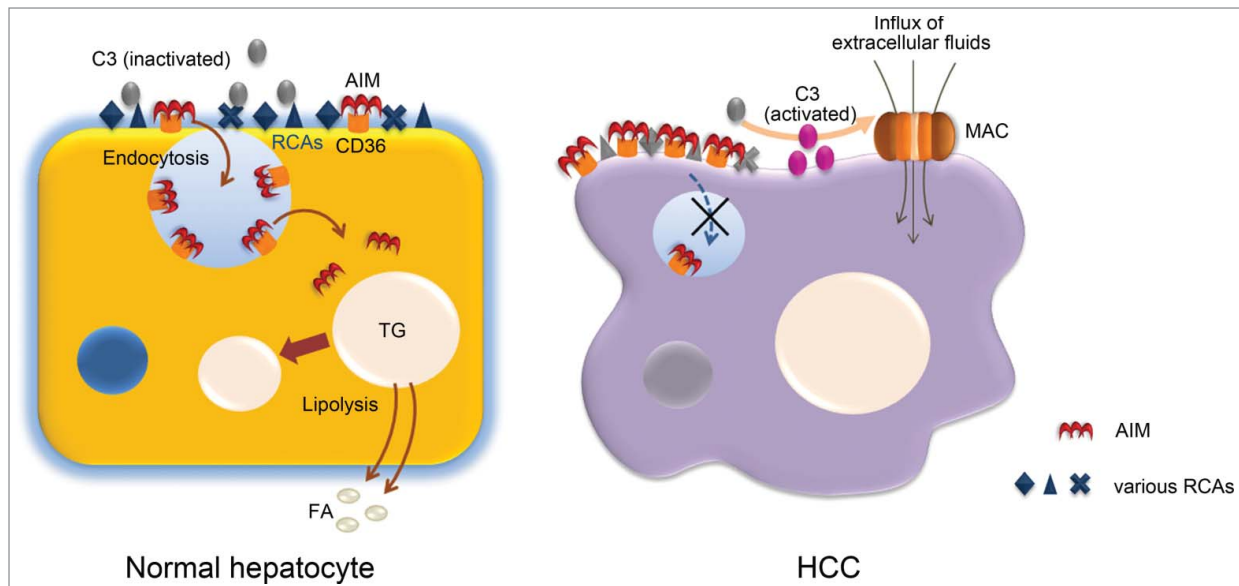
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**Figure 1.** Protective effects of AIM in liver steatosis and hepatocellular carcinoma. *Left:* Circulating AIM is incorporated into normal hepatocytes and induces lipolysis, thereby decreasing triacylglycerol (TG) storage within the cells. This response prevents the progression of liver steatosis. *Right:* In contrast, as a result of defective endocytosis, AIM accumulates on the surface of hepatocellular carcinoma (HCC) cells. Extracellular AIM reduces the regulatory activity of regulators of complement activation (RCAs) through direct binding, resulting in activation of the complement cascade (activation of C3 and formation of MAC) directed against the AIM-bound HCC cells. This leads to necrotic death of HCC cells, thereby preventing the development of liver tumors. C3, complement component 3; FA, fatty acid; MAC, membrane attack complex.

development. Indeed, in contrast to wild-type mice, in which HCC tumors seldom develop after HFD-induced severe steatosis, all *AIM*<sup>-/-</sup> mice bear multiple tumors when fed an HFD for a year.<sup>1</sup> This HCC tumor prevention by AIM appears to be specifically due to AIM-induced cell death because *AIM*<sup>-/-</sup> and wild-type mice show comparable grades of HFD-induced inflammation and fibrosis in the liver, which are currently recognized as an important basis for liver carcinogenesis. Thus, AIM may not affect hepatocyte carcinogenesis originating from advanced liver steatosis in a manner similar to non-alcoholic steatohepatitis in humans,<sup>8</sup> but may instead prevent HCC tumor development through the elimination of cancerous hepatocytes. It is worth emphasizing that normal hepatocytes are not killed because they incorporate AIM, thus preventing extracellular accumulation.

Extracellular AIM does not provoke signaling cascades, but instead induces necrotic death in HCC in an unexpected manner—it hijacks the complement cascade, an ancient immune defense against invading pathogens, to kill undesirable self cells. In contrast to bacteria, normal

and cancerous mammalian cells are protected from complement attack by the expression of multiple regulators of complement activation (RCAs)<sup>9</sup> such as CD55, complement regulator complement receptor 1-related gene/protein-y, complement factor H, and CD59. These molecules are analogous to the camouflage that hides targets from aerial bombardment. AIM figuratively demolishes the camouflage, exposing the target to the attacker. This effect appears to be brought about by the direct binding of AIM to RCA molecules, similar to the AIM-mediated reduction of FASN in normal hepatocytes and adipocytes. However, the precise mechanism by which AIM association reduces the regulatory activity of RCAs remains unresolved.

Of course, the next step for this research is to apply it to HCC therapy. Although we have demonstrated a strong preventive effect of AIM for HFD-induced HCC development, only a partial therapeutic effect of recombinant AIM (rAIM) administration was observed for existing large HCC tumors. Thus, several concerns remain. First, the amount of rAIM that should be administered

remains to be determined, and the route of administration must be optimized. In mice, most of the intravenously injected rAIM is incorporated into normal cells before reaching the region of HCC, and a large amount of protein is therefore required. Mesenchymal or portal vein injection could result in more efficient transfer of rAIM to HCC tumors. Similarly, direct injection of rAIM into the hepatic artery, as used for some anticancer drugs, may be most effective in humans. These administration routes might also reduce the amount of therapeutically required rAIM. Second, to increase the amount of rAIM that collects on the surface of HCC cells, it might be worth considering the application of certain drug delivery systems (DDSs) combined with specific tumor-targeting antibodies. We currently do not know whether AIM is effective at eliminating cancer cells other than HCC; AIM may only specifically accumulate on cancer cells derived from cells that would normally incorporate AIM, and thus may effectively kill only these cell types. However, the application of a specific AIM-DDS may increase the variety of targetable cancer cell types.

In addition, we propose that evaluation of AIM blood levels might help to predict HCC susceptibility. In another report, published during the same period as the one in *Cell Reports*, we demonstrated that circulating AIM levels increase with the progression of liver damage in human patients.<sup>10</sup> Thus, it is plausible that patients whose blood AIM levels do not increase to a level sufficient to eliminate cancerous hepatocytes may develop HCC tumors. A prospective cohort study of HCC development in patients with

comparable levels of liver damage is needed to assess this possibility, but we hope that AIM might have value as a risk marker of HCC development and that AIM administration might be a preventive therapy for HCC.

In summary, the potent anti-HCC protective effect of AIM demonstrated by our study suggests that AIM could form the basis of next-generation therapeutic strategies for cancer.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed

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