Burning Fat to Fuel EVs

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Obesity is one of the most prevalent metabolic diseases worldwide, and it increases the risk of developing type 2 diabetes, heart failure, cancer, and other diseases (1). White adipocytes are specialized cells that store enormous amounts of lipids, predominantly in the form of triglycerides, and they release free fatty acids into the circulation in response to lipolytic stimuli. In addition, adipocytes communicate with other cells by secreting a complex set of hormones, cytokines, and metabolites that maintain white adipose tissue (WAT) homeostasis and regulate the function of distant organs, such as the heart, liver, pancreas, and brain (2). In obesity, shifts in adipocyte-derived signals contribute to the development of local and systemic inflammation, pathologic tissue remodeling in WAT, and adverse metabolic sequelae such as insulin resistance (3,4). Increasing our understanding of how adipocytes communicate with neighboring and distant cells may reveal new therapeutic targets to treat obesity or associated metabolic diseases.

An emerging body of literature indicates that adipocytes release extracellular vesicles (EVs) that contain a wide variety of cargo, including lipids, proteins, nucleic acids, and even organelles such as mitochondria (5–8). These EVs mediate cross talk between cells within WAT but can also exert systemic effects on distant organs. For example, adipocyte-derived extracellular vesicles (AdEVs) can either impair or enhance insulin sensitivity in hepatocytes and muscle cells (9,10), and they can also increase pancreatic β-cell production of insulin (11). However, the mechanisms that regulate the release of EVs by adipocytes are not fully understood.

In this issue of *Diabetes*, Huang et al. (12) report that lipolysis leads to activation of the DNA repair enzyme p53 to stimulate release of AdEVs. They demonstrate that the lipolysis-inducing compounds forskolin and isoproterenol lead to the release of EVs by 3T3-L1 adipocytes. Interestingly, this process was not EV specific, as inducing lipolysis also stimulated the release of many free proteins, such as fatty acid binding protein 4 (FABP4). Secretion of free proteins and AdEVs is suppressed by the p53 inhibitor pifithrin-α or when expression of p53 is reduced by shRNA-mediated knockdown. Consistent with this result, serum from p53-deficient mice had reduced EV particle counts and decreased levels of FABP4. Gain-of-function studies showed that activation of p53 led to the release of more AdEVs and FABP4 from adipocytes. Nutlin, a compound that indirectly activates p53, also led to the release of more AdEVs. Since p53 is classically involved in DNA damage repair, the authors used doxorubicin to induce DNA damage and found that this treatment led to the release of more AdEVs in a p53-dependent manner. Interestingly, in mice with ERCC1 haploinsufficiency, which causes increased DNA damage that goes unrepaired, p53 expression is upregulated, and this is associated with increased FABP4 in serum in male but not female mice, a sex dependency that warrants further investigation. Overall, these studies indicate that lipolysis activates p53 to induce release of AdEVs in vitro and in vivo (Fig. 1), and the findings are consistent with those from prior studies showing that diet-induced obesity is associated with increased DNA damage, p53 activation, and release of proinflammatory factors that promote WAT inflammation and insulin resistance (13).

The mechanisms by which p53 regulates the release of AdEVs are not yet clear. As was shown previously in HeLa cells (14), the authors identified a potential role for mammalian target of rapamycin (mTOR). They showed *1*) that p53 activation with nutlin or doxorubicin inhibits mTOR activity and S6 phosphorylation at serines 240/244 and 235/236 and *2*) that inhibition of mTOR complex 1 (mTORC1) with torin or rapamycin leads to increased release of AdEVs. While numerous studies have identified how p53 and genotoxic stress regulate the activity of mTORC1 (15), it is not clear how mTORC1 regulates the production or release of AdEVs. It has been shown that

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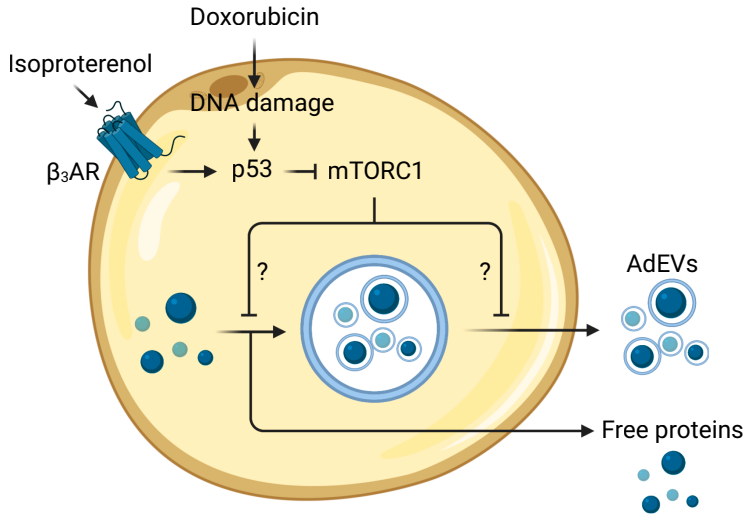

Figure 1—Lipolysis and DNA damage promote the release of EVs by adipocytes in a p53-dependent manner. Lipolytic stimuli activate p53 and promote the release of lipid-rich AdEVs. DNA damage and pharmacologic activation of p53 suppress the activity of mTORC1, which relieves the inhibition on free protein release and the packaging and/or release of AdEVs. The resultant AdEVs contain various types of cargo, including cytosolic, nuclear, and mitochondrial proteins. Conversely, genetic deletion or inhibition of p53 leads to impaired release of AdEVs and other secreted free proteins. The mechanisms by which mTORC1 regulates p53-dependent release of adipocyte-derived proteins and EVs are not yet well defined. β_3AR, β-3 adrenergic receptor. Created with BioRender.com.

mTORC1 physically interacts with Rab27a, a small GTPase that is required for exosome secretion (14); however, Rab27a function is only one part of the highly complex and coordinated process of EV biogenesis. It was demonstrated that the AdEVs released after lipolysis tend to be 130–400 nm in diameter, with predominant EV subsets that are 130, 180, 331, and 380 nm, on average. These subsets contain a diverse set of proteins that lack secretion signals and are typically localized to the cytoplasm and organelles such as the nucleus and mitochondria. However, AdEVs released after p53 activation tend to be smaller. This result suggests the p53-mTORC1 pathway may regulate the release of a subset of smaller AdEVs and that there are likely other pathways that regulate the release of larger AdEVs.

There are many questions that remain unanswered about the functional relevance of AdEVs released in response to lipolytic stimuli. One open area for investigation is whether lipolysis and p53 activation lead to selective packaging of contents into AdEVs. Huang et al. (12) use only a small number of markers, such as TSG101 and CD63, to characterize AdEVs, but they provide proteomic data on conditioned media, indicating that AdEVs can contain numerous proteins, including those localized to mitochondria. Whether p53 is involved in packaging certain types of cargo, such as mitochondria, within AdEVs prior to release is a particularly interesting question in light of recent studies that report that AdEVs containing oxidatively damaged mitochondria are delivered to tissue-resident macrophages for degradation (16) but can also be released into the circulation for delivery to distant organs, such as the heart, to protect against ischemia-reperfusion injury (5,17). While these observations suggest that AdEVs, including those that contain mitochondrial components, can have beneficial effects locally and systemically, in some circumstances AdEVs can contribute to pathology in obesity (18). It remains unknown whether the lipolysis-p53-mTORC1 pathway identified by Huang et al. (12) regulates the release of specific subsets of AdEVs, whether this pathway regulates AdEV production in the endo-lysosomal system or at the plasma membrane, or whether these AdEVs confer beneficial and/or deleterious effects on WAT or on distant organs. Further research into these topics may reveal previously unknown biological pathways that can be targeted therapeutically to treat metabolic diseases such as obesity.

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