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Review

Complex regulation of autophagy in cancer – Integrated approaches to discover the networks that hold a double-edged sword

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ABSTRACT

Autophagy, a highly regulated self-degradation process of eukaryotic cells, is a context-dependent tumor-suppressing mechanism that can also promote tumor cell survival upon stress and treatment resistance. Because of this ambiguity, autophagy is considered as a double-edged sword in oncology, making anti-cancer therapeutic approaches highly challenging. In this review, we present how systems-level knowledge on autophagy regulation can help to develop new strategies and efficiently select novel anti-cancer drug targets. We focus on the protein interactors and transcriptional/post-transcriptional regulators of autophagy as the protein and regulatory networks significantly influence the activity of core autophagy proteins during tumor progression. We list several network resources to identify interactors and regulators of autophagy proteins. As *in silico* analysis of such networks often necessitates experimental validation, we briefly summarize tractable model organisms to examine the role of autophagy in cancer. We also discuss fluorescence techniques for high-throughput monitoring of autophagy in humans. Finally, the challenges of pharmacological modulation of autophagy are reviewed. We suggest network-based concepts to overcome these difficulties. We point out that a context-dependent modulation of autophagy would be favored in anti-cancer therapy, where autophagy is stimulated in normal cells, while inhibited only in stressed cancer cells. To achieve this goal, we introduce the concept of regulo-network drugs targeting specific transcription factors or miRNA families identified with network analysis. The effect of regulo-network drugs propagates indirectly through transcriptional or post-transcriptional regulation of autophagy proteins, and, as a multi-directional intervention tool, they can both activate and inhibit specific proteins in the same time. The future identification and validation of such regulo-network drug targets may serve as novel intervention points, where autophagy can be effectively modulated in cancer therapy.

1. Introduction

Most mutations affecting the integrity of signaling pathways and cellular processes display either pro- or anti-oncogenic effects. Autophagy (cellular self-degradation) is usually considered as a tumor-suppressing mechanism, though it can also enable tumor cell survival upon stress, and may promote metastasis formation. Thus, it is not obvious which therapeutic approaches can modulate autophagy in the desired way. Here, we show that systems-level knowledge is needed to select efficient anti-cancer drug targets that affect autophagy. Multi-target drugs and combination therapies may become more effective than previous autophagy-related monotarget approaches.

Macroautophagy involves the sequestration of cytosolic material into double membrane vesicles termed autophagosomes for delivery to the lysosome, where the cargo is degraded by acidic hydrolase enzymes [1]. Autophagy is a key response mechanism to numerous extracellular and intracellular stresses [2]. These include, for example, nutrient and growth factor deprivation and hypoxia. Under starvation, the enhanced autophagic activity provides the cells with metabolic intermediates to meet their bioenergetic

Abbreviations: ERK, extracellular signal regulated protein kinase; FoxO1/3, forkhead family transcription factor; GSK3, glycogen synthase kinase-3; HIF, hypoxia-inducible factor; IGF, insulin-like growth factor; IRE1, inositol-requiring protein 1; JNK, c-Jun N-terminal kinase; NF-κB, nuclear factor kappa beta; NRF2, nuclear factor erythroid 2-related factor 2; PKA, protein kinase A; p53, TP53 tumor suppressor protein; RAS, small GTPase protein; SREBP, sterol regulatory element-binding protein; TFEB, transcription factor EB; TGF-β, transforming growth factor beta; WNT, wingless and int-like protein.

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demands [2]. Autophagy is the only cellular catabolic process that can eliminate damaged or reactive oxygen species (ROS)-overproducing mitochondria, and thereby limit general oxidative damage [2].

Autophagy is regulated by conserved upstream signaling pathways integrated by the mammalian kinase target of the immunosuppressant rapamycin (mTOR) [1]. Available nutrient or growth factors activate the insulin/IGF-1 – TSC – TOR signaling axis, which inhibits autophagy, and stimulates cell growth and proliferation. Nutrient or growth factor limitation, hypoxia and other cellular stressors are known to deactivate this signaling system, leading to autophagy induction and suppression of cell growth and proliferation [3]. Several other pathways (including RAS/PKA, RAS/ERK, IRE1/JNK, TGF-β, WNT/GSK3, HIF) and transcription factors (TFs), such as NRF2, FoxO and p53 have also been described to effect autophagy [4,5]. Interestingly these signaling pathways are also important in cell growth, proliferation, angiogenesis, immunity, cell survival and cell death [6], functions whose alteration are listed among the hallmarks of cancer [7]. Thus, these data show that the control of autophagy is affected during tumorigenesis.

Numerous studies examined the role of autophagy in cancer, but the results are quite ambiguous. On the one hand, autophagy has tumor suppressing functions by (a) suppressing chromosomal instability and therefore preventing the accumulation of oncogenic mutations; (b) restricting oxidative stress, which is also an oncogenic stimulus; (c) promoting oncogene-induced senescence, and (d) reducing intratumoral necrosis and local inflammation [2,8,9]. On the other hand, enhanced autophagy represents a prominent mechanism used by tumor cells to escape from hypoxic, metabolic, detachment-induced and therapeutic stress as well as to develop metastasis and dormant tumor cells [2,8,9]. During tumorigenesis, autophagy is frequently switched on and off, resulting in highly regulated anti- and pro-tumorigenic effects. Therefore, autophagy can be considered as a double-edged sword during tumorigenesis [10]. As autophagy is switched on and off during tumorigenesis, we can assume that it is not the autophagic machinery itself but the protein–protein interaction and regulatory networks that continuously is changing during tumor progression. These networks can context-dependently control the mechanism of autophagy (Fig. 1).

In the following sections, we briefly review protein–protein and regulatory network resources to examine autophagy on the systems-level. Then, we summarize frequently used *in vivo* genetic models, forward and reverse genetics-based methods, as well as fluorescence techniques to experimentally study autophagy and validate the systems-level predictions of network analysis. Finally, we present the challenges and possibilities of network pharmacological approaches to modulate autophagy in cancer.

2. Interactors of autophagy proteins

Currently, several databases describing protein–protein interactions (PPI) exist, but only few of them contain sufficient information on autophagy-related proteins. We benchmarked six well-known, general PPI resources and two autophagy-specific network databases to analyze the presence of a core set of 38 autophagy components (listed in Table 1). With this comparison we pointed out the number of autophagy components and their interactions in various resources (Table 2).

We selected major PPI resources where the experimental/literature source of the given interaction is listed allowing the users to check and examine the details of the interactions. We examined three PPI databases that contain manually curated interaction data: (1) the Human Protein Reference Database (HPRD) [11]; (2) the IntAct resource [12]; and the Molecular INTeraction database (MINT) [13]. From these databases, IntAct represents the highest number of core autophagy components (36 of the 38) and interactions (2702). We also examined two PPI resources that contain more interactions gained from high-throughput screens: (1) the Search Tool for the Retrieval of Interacting Genes (STRING) [14] and (2) the Biological General Repository for Interaction Datasets (BioGRID) resource [15]. In STRING, there were interaction data (335 PPIs) for 37 of the 38 autophagy core proteins but BioGRID contained more interactions (641 PPIs for 36 proteins). In addition, we examined the Interologous Interaction Database (I2D) containing the mostly predicted PPIs [16]. I2D has 10,182 PPIs for 37 autophagy core proteins. Note that most of these PPIs are inferred based on orthology, and the original experimental evidences were coming from mainly high-throughput screens. Despite the fact of the potential high number of false positive PPIs, I2D could serve as an efficient pool of possible autophagy-related interactions. Further filtering and experimental validation could point out true positive interactions in given experimental contexts.

We also examined interaction databases focusing specifically on autophagy. To our knowledge, there are only two such databases: the Autophagy Database (ADB) and the Autophagy Regulatory Network (ARN). ADB contains a lot of different information on the components of the autophagic process [17]. ADB includes 28 proteins from the 38 core autophagy components

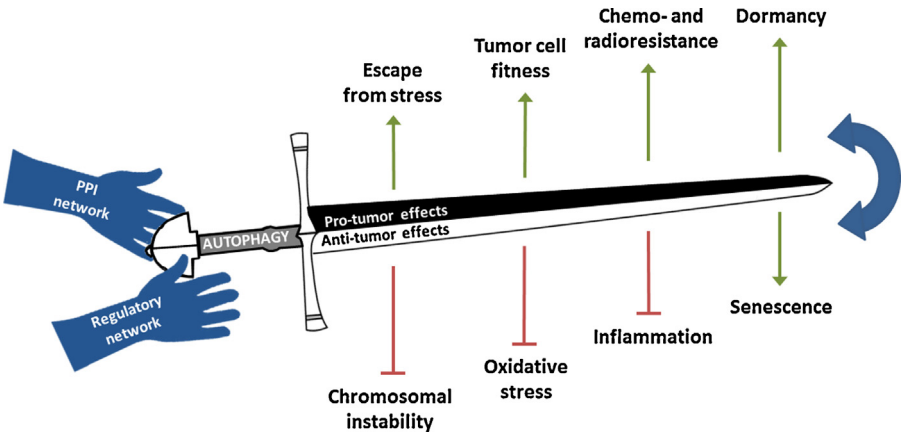

Fig. 1. Autophagy as a double-edged sword in cancer biology. Autophagy has both pro-, and anti-tumor effects. Autophagic activity is precisely regulated and continuously switched on and off during the phases of tumorigenesis. This mechanism is carried out by the protein–protein interaction (PPI) network containing protein interactors (e.g., enzymes and adaptors) of autophagy proteins as well as by regulatory networks of transcription factors and miRNAs. Coordinated action of these networks controls the activity of autophagy in cancer. For a detailed network view of this connection, see Fig. 2.

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