# Relevance of Autophagy in Cancer Stem Cell and Therapeutic

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**Abstract**

Autophagy in cancer acts as a double-edged sword whose functional discrepancies precisely depend on cancerization, progression, and type. During stress, they promote cancer cell survival, induce carcinogenesis due to their accumulated genetic mutations or abnormal cell signaling, initiating fast replication capacity, promoting more aggressiveness, and resistant to programmed cell death. Consequently, the study has drawn focus on autophagy in cancer. However, convincing preclinical and clinical evidence on the cytoprotective in addition to the lethal roles of autophagy for cancer stem cells (CSCs) are missing. There are quite a lot of clinical trials ongoing to manipulate autophagy and in this manner decide the result of disease therapy. The clinical relevance of this work encompasses autophagy modifiers, such as rapamycin and chloroquine that control autophagy in anticancer therapy, since autophagy plays roles in both tumor suppression and promotion. Further detailed examination of autophagy in cancer is required to understand how an increased function of autophagy in the tumor microenvironment, stemness, migration and invasion, dormancy, and drug resistance could be tweaked for enhanced therapeutic benefit by eradicating minimal residual disease and preventing metastasis. Here, we recapitulate how autophagy modulates the therapeutic potential to exterminate CSCs.

**Keywords**

Cancer stem cells · Self-renewal · Autophagy · Mitophagy · Anticancer therapy

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## 10.1 Introduction

Pluripotent cancer stem cells (CSCs) are subset of cancer cells that accentuate their ability to self-renew and (Aponte and Caicedo 2017) differentiate into all somatic cell lineages by indefinite cell division giving rise to the heterogeneous tumor populations and maintain their undifferentiated state (Liu et al. 2013). When a very small population of CSCs was introduced into an immunocompromised mice, it initiated the formation of the original tumor (Ghiaur et al. 2012). They are phenotypically slow cycling and their self-renewing capacity is accountable for tumor growth, resistance to therapy, and recurrence after treatment.

Autophagy is a double-edged sword in the progression of neoplasia and has further produced immense hurdles for researchers to explore its impression on carcinogenesis and tumor development. It has labeled tumor-suppressive and tumor-promoting functions (White and DiPaola 2009). Cytoprotective role of autophagy prevents malignant transformation through the ability to empower the premalignant cells by efficiently meeting up with the increased energy requirements by recycling cellular components that are important in maintaining the physiological tissue homeostasis. This attribute propagates their accommodation within the stress (metabolic, genotoxic, and inflammatory) occurring after the malignant transformation induced in response to anticancer (chemo/targeted/radiotherapy) treatment. Stresses including nutrient and energy stress, ER stress, danger-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs), hypoxia, redox stress, and mitochondrial damage induce autophagy, alongside EMT and stemness. The cytoprotective role of autophagy can turn into a cell-suicidal weapon causing cell death in cancer cells. Defective autophagy has been linked with increased oncogenesis. For instance, low expression of Beclin-1 (Atg6) in some types of cancers of the prostate, breast, and ovary because of monoallelic mutations (Qu et al. 2003). However, the presence of heterozygosity in mice for the *beclin-1* gene makes it cancer prone (Qu et al. 2003; Yue et al. 2003) due to absence of functional of Beclin-1.

Cancer progression shows a degree of dependency on the existence of CSCs. The role of autophagy in cancer is multifaceted and has been studied extensively. High levels of autophagy contribute to pluripotency of CSCs in other cancer types, including colorectal cancer (Kantara et al. 2014), pancreatic cancer (Rausch et al. 2012; Viale et al. 2014), glioblastoma (Galavotti et al. 2013), chronic myeloid leukemia (Bellodi et al. 2009), and bladder cancer (Ojha et al. 2016). Despite recent advancement in research, the underlying molecular mechanism inducing autophagy in CSCs remains to be determined. It is difficult to explain how autophagy promotes stemness, have been preserved across different cancer. Mitophagy is a selective autophagy that unambiguously plays an important role in the quality control and homeostasis of mitochondria. Mitochondrial functional pathways play a crucial role in a vital interaction between cancer cells and stromal cells for cancer cell initiation, progression, and treatment response. They emanate a profound role in sustaining CSCs in adverse conditions and initiating their metabolic reprogramming to support the increased bioenergetic demand of the tumor. Transcription factors like SMAD

(Nazio et al. 2019), NF-Kb (Zhang et al. 2016), MITF (Moller et al. 2019), STAT3 (Marcucci et al. 2017; Zhang et al. 2016), FOXO (Naka et al. 2010), ATF4 (Pallmann et al. 2019), NANOG (Liu et al. 2017), regulate autophagy and mitophagy in the induction of EMT and maintenance of CSCs. Like autophagy, mitophagy acts in cancer as bimodal processes. Unfortunately, there are unanswered roles of canonical autophagy in cancer (Gewirtz 2014). Therefore, does mitophagy has a role in cancer? CSCs play an unbiased role in promoting therapy resistance leading to tumor recurrence (Shibue and Weinberg 2017), and autophagy deliberately endorses disseminated tumor cells (DTCs) which further lead to the metastatic expansion of tumors (Sosa et al. 2014). To understand how autophagy and mitophagy can inhibit to repress both the above phenotypes are challenging task for translational cancer. Recent studies have linked CSCs with chemoresistance and cancer relapse, autophagy, mitophagy, and CSCs showcase novel perspectives on potential therapeutic targets for enhancing anticancer drug sensitivity. The study of autophagy in cancer has been therapeutically manipulated by many investigators and various clinical trials that are already ongoing to regulate the result of disease therapy.

## 10.2 Autophagy/Mitophagy Drives Cancer Stem Cells Fate

CSCs are a heterogeneous population; they escalate tumor growth and progression by accelerating the proliferative potential and constitute a source for recurrence of cancer. Functional properties of cancer cells are influenced by epigenetic, genetic, and microenvironmental factors. To proliferate in its microenvironment, CSCs have a functional correlation with autophagy and mitophagy. Autophagy, a catabolic pathway enables CSCs to show autophagy dependence and may act as an oncosuppressive depending on tumor stage and type. They exploit the pro-survival attribute of autophagy at the later stage of oncogenesis to meet up with high-energy demands by a supply of metabolites. ATG-encoded gene products play a significant role in CSCs of numerous cancers. Beclin 1/Atg6 modulates CSC plasticity and tumorigenesis in vivo. However, in different cancers, Beclin 1 acts as a tumor suppressor, like human prostate, breast, and ovarian tumors (Liang et al. 1999; Qu et al. 2003; Shen et al. 2008). Improved survival in patients is observed having high Beclin 1 levels affected by large B-cell lymphoma, high-grade gliomas, or hepatocellular carcinoma (Ding et al. 2008; Huang et al. 2011; Pirtoli et al. 2009). The stemness was augmented by the transformation of $CD133^{-}$ to $CD133^{+}$ cells due to the inhibition of mTOR affecting the liver tumor cells by interrupting the differentiation and stimulating the tumor development in vivo (Yang et al. 2011). Suppression of autophagy by knockdown of autophagic proteins Atg5 and Atg7, curtails stemness markers, such as Sox2, Nanog, and Oct4, resulting colorectal CSCs to undergo suppressed cell proliferation and improved cell senescence (Sharif et al. 2017). In colorectal cancers, mutations in Atg5, Atg12 have been described (Kang et al. 2009) while deletion of Atg5 or Atg7 is supporting the advancement of liver hepatomas (Takamura et al. 2011). Autophagy induction by overexpressing Atg4A

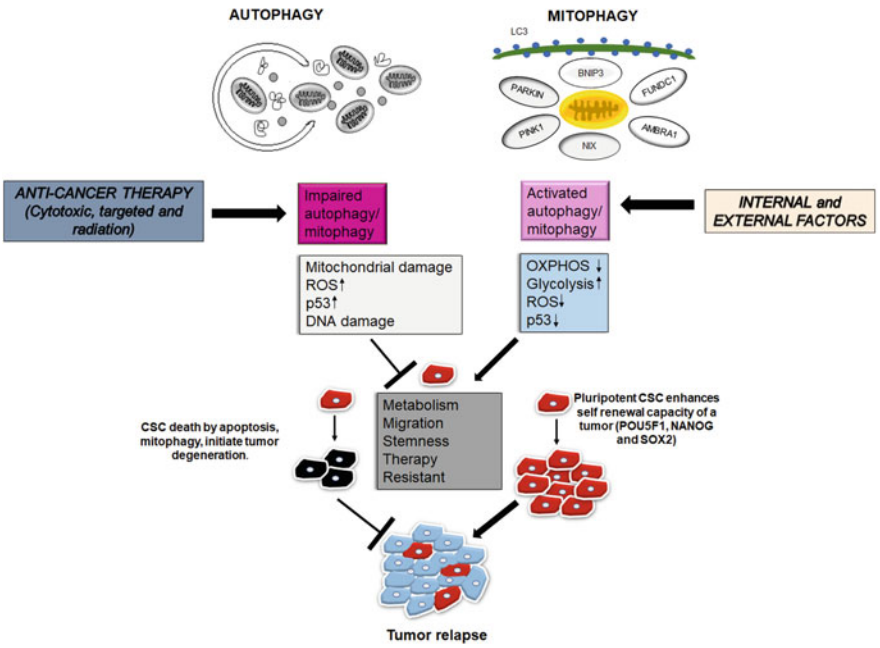


**Fig. 10.1** The basal level autophagy and mitophagy are important for cell metabolism. When there is stress due to anticancer therapy, autophagy, and mitophagy get impaired, while they are activated due to internal and external factors leading to either suppression or progression of cancer

protein promotes mammosphere formation and hence increases CSC numbers and in vivo tumorigenesis (Wolf et al. 2013). The conditional knockout of Atg3 affected the continued existence of CML cells and leukemogenesis (Altman et al. 2011). Inhibition of Atg4B resulted in its increased phosphorylation followed by arresting the tumor growth in animal models in a subset of glioblastoma cancer (Huang et al. 2017). The depletion of ATG4B impaired the survivability of CML stem/progenitor cells (Rothe et al. 2014). Knockout of Atg4C in mice increased the propensity to develop fibrosarcomas induced by methylcholanthrene, hence play a tumor-suppressor role. Contrastingly, its tumorigenic role in breast cancer was delineated (Antonelli et al. 2017). Tumor suppressive role of Atg4D expression was observed in colorectal carcinogenesis (Gil et al. 2018). Moreover, its tumor-promoting role was highlighted when cancer cells were sensitized to chemotherapeutic drugs on ATG4D silencing (Betin and Lane 2009) (Fig. 10.1).

EMT (epithelial to mesenchymal transition) signaling is an important characteristic of CSCs (Shibue and Weinberg 2017). Autophagy signaling is strongly correlated to EMT in enhancing the metastatic potential of CSCs to migrate by maintaining their mesenchymal signature in the later stages of metastasis. Interestingly, during early metastasis autophagy decreases the invasion and migration of tumor cells in situ. In glioblastoma cells, blocked cell migration and invasion were

caused by nutrient deprivations and mTOR inhibition (Catalano et al. 2015). Using specific siRNAs directed against the autophagy-related factors DRAM1 and p62 proteins, autophagy-controlled bioenergetic metabolism, migration/invasion of glioblastoma CSCs was thwarted while the mesenchymal phenotype was restored on autophagy upregulation (Galavotti et al. 2013). Furthermore, in glioblastoma cells, enhanced migration and invasion with EMT regulators continued with knockdown of Beclin 1, Atg5, and Atg7 (Catalano et al. 2015). EMT promotes stemness and can give rise to CSCs through the core stemness factors POU5F1, Sox2, and Nanog, including Slug and Twist that maintains the pluripotency of CSCs and tumor-propagating properties (Mani et al. 2008). Hypoxia and TGF-β through MITF (Caramel et al. 2013), Sox2, and Nanog (Sharif et al. 2017) promote EMT via activating autophagy. Autophagy may promote tumor cell dormancy, lipid metabolism, mitochondrial function, and CSCs existence in muscle stem cells and HSCs (Ho et al. 2017; Warr et al. 2013). It ensures a reversible dormant pool of CSCs potentially making a contribution to tumor repopulation and preventing irreversible senescence (Ho et al. 2017). Autophagy plays a decisive role in the survival of disseminated tumor cells (DTCs) at secondary location to establish drug resistance, minimum residual disease, and metastatic dormancy (Sosa et al. 2014). Interestingly, these DTCs are CSCs that are relatively quiescent and motile state expressing upregulated CSC markers in the bone marrow of breast cancer patients (Balic et al. 2006). Furthermore, a selective form of autophagy known as mitophagy promotes stemness. It abrogates senescence by disrupting the ROS-induced DNA damage and has a principal role in maintaining the stem cell population renewal and homeostasis. It has been reported to maintain hepatic CSCs by regulating p53 localization. Therefore, inhibition of mitophagy phosphorylates p53 by PINK1 leading to its translocation to the nucleus where Oct4 and Sox2 induction of Nanog get alienated. Mitophagy evokes CSCs dependence more on glycolysis for energy needs and hence contributes to its quiescent state. Recent evidence suggests that mitochondrial dysfunction also encourages oncogenesis (Boya et al. 2018). Mitochondrial ROS due to BNIP3 loss subsequently resulted from defects in mitophagy followed by mammary neoplastic progression to metastasis (Chourasia et al. 2015) (Table 10.1).

## 10.3 Targeting Autophagy/Mitophagy: New Therapeutic Strategies

CSC generation, differentiation, plasticity, migration/invasion, and immune resistance are very much dependent on the variation of autophagy/mitophagy. During anticancer therapy, CSCs remain at the dormant stage to cope with intracellular and environmental stress, involving oxidative stress triggered by overproduction of reactive oxygen species (ROS). These dormant cells arise from EMT tumor cells and become non-cycling autophagic CSC which are later maneuvered on the release of paracrine factors (like MET, TGF-β receptor, IL-6 receptor, PDGFR, EGFR, FGFR, Hedgehog/Smoothened, WNT/Frizzled, Gas6/AXL, and Notch ligands) to

**Table 10.1** Role of autophagy in different types of cancer and genes targeted for anticancer therapy

| Types of cancer | Animal model/ cells/CSCs | Autophagy as protective or lethal or both | Targeted genes involved in the induction of autophagy | References |
|---|---|---|---|---|
| Neuroblastoma, multiple myeloma cells | SH-SY5Y cells | Protective | NAMPT | Billington et al. (2008), Cea et al. (2012), Ghosh and Matsui (2009), Schneider et al. (2011), Sharif et al. (2017) |
| Colorectal cancer | HCT116, HT29, CaCO2, and DLD1CSCs; DCLK1-positive colon CSCs | Protective | Endolysosomal RAB5/7 regulating mitophagic pathway; LC3, Beclin1, Atg6 | Kantara et al. (2014), Takeda et al. (2019) |
| Malignant pluripotent embryonal carcinoma | NT2/D1 CSCs | Protective | NAMPT | Sharif et al. (2017) |
| Breast cancer | MCF-7 CSCs; SUM149 CSCs | Both protective and lethal in MCF-7 and protective in SUM149 | Protective: Beclin1, c-Jun NH2 terminal kinase (JNK/SAPK) in MCF-7, and Atg4A in SUM149 Lethal: Beclin1, Akt/mammalian target of rapamycin (mTOR) pathway in MCF-7 | Protective: MCF-7 (Chaterjee and van Golen 2011; Sanchez et al. 2011) and SUM149 (Wolf et al. 2013) Lethal: MCF-7 (Liang et al. 1999; Lu et al. 2014) |
| Prostate and breast cancer | PC-3 and DU145 cells; MDA-MB-231 cells | Lethal | AMP-associated protein kinase (AMPK)/Unc-51 like autophagy activating kinase 1 (ULK1) pathway and inhibition of mTOR/Raptor complex 1 expression | Aryal et al. (2014) |

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**Table 10.1** (continued)

| Types of cancer | Animal model/ cells/CSCs | Autophagy as protective or lethal or both | Targeted genes involved in the induction of autophagy | References |
|---|---|---|---|---|
| Pancreatic cancer | $CD133^{+}$ pancreatic CSCs, BxPc-3 ($CSC^{low}$) and MIA-PaCa2 ($CSC^{high}$), inducible mouse model of mutated *Kras* | Protective | HIF-1a; Beclin1, Atg4B, LC3, p62; AMPK, LC3 | Rausch et al. (2012), Viale et al. (2014), Zhu et al. (2013) |
| Urinary bladder cancer | T24 and UM-UC-3 CSCs; T24 CSCs | Protective | Beclin1, Atg7, and p62; IFN-γ-mediated JAK2 and STAT3 pathway | Ojha et al. (2014), Ojha et al. (2016) |
| Brain tumor | CSCs: MDNSC11, MDNSC13, MDNSC23, MDNSC16; GBM stem cells–GSCs | Lethal and protective | p16INK4/Rb pathway, Atg5; DRAM1, SQSTM1, p62 | Galavotti et al. (2013), Jiang et al. (2007) |
| Chronic myeloid leukemia | $p210^{BCR/ABL}$-expressing CML cells, CML lymphoid BC cell line BV173, K562 cells | Protective | LC3, Atg5, Atg7 | Bellodi et al. (2009) |

cycling CSC with low autophagy. Thus, autophagy and mitophagy enable CSCs to colonize, migrate and metastasize, defy apoptosis and antitumor drugs and hence become therapy-resistant by its self-renewal property and replace the pool of differentiated tumor cells (Marcucci et al. 2017) (Table 10.2).

Autophagy/mitophagy has an inevitable role in cancer cell survival, metastasis, and therapy resistance. The potentially new targeted therapeutic strategy is to use double or triple combinatorial doses of drugs or antibodies and/or radiation to modulate autophagic machinery to efficiently eradicate CSCs. Chemotherapy is a widespread treatment strategy for cancer therapy that engulfs dividing cells and disrupts cancer–cell division. However, several studies have revealed that the overall success rate of chemotherapy is often restricted via the upregulation of cytoprotective activation of autophagy in CSCs which protects cancer cells subjected to anticancer therapy. Cancer chemotherapeutic drugs 5-Fluorouracil (5FU) and cisplatin used in various solid cancers, like, gallbladder and colorectal cancers show autophagy-regulated chemoresistance (Ferreira et al. 2016; Liang et al. 2014;

**Table 10.2** Anticancer therapy, targeted autophagy induced molecular pathway, and their current clinical status

| Anticancer therapy | Type of cancer | Molecular pathway targeted | Current status | References |
|---|---|---|---|---|
| Liensinine, an extracted from the seed embryo of *Nelumbo nucifera* Gaertn | Breast cancer cells (MDA-MB-231 and MCF-7) | Inhibition of autophagosome–lysosome fusion | Preclinical trials | Zhou et al. (2015) |
| Resveratrol, a natural phenol | Breast CSCs (MCF-7 and SUM159) | Suppression of the Wnt/β–catenin signaling pathway | Preclinical trials | Fu et al. (2014) |
| Mefloquine, an anti-parasite used to treat malaria | Colorectal cancer cell lines (HCT116, HT29, $CaCO_2$, and DLD1) | Inhibited lysosomal activity by targeting RAB5 and RAB7 resulted in suppression of mitophagic PINK1/PARKIN | Preclinical trials | Takeda et al. (2019) |
| Metformin, used for the treatment of type 2 diabetes | Pancreatic intraepithelial neoplasia (PanIN) | Modulating the mTOR signaling pathway | Preclinical trials | Mohammed et al. (2013) |
| Rottlerin, a polyphenolic compound | Metastatic colorectal cancer cell lines (Tu12, Tu21, and Tu22 cells) breast CSCs, prostate CSCs (human prostate tumor samples) | mTOR inhibition; AMPK activation and proteasome inhibition; AMPK activation and inhibition of PI3K/Akt/mTOR pathway | Preclinical trials; preclinical trials; clinical + preclinical trials | Francipane and Lagasse (2013), Kumar et al. (2013, 2014) |
| Combinatorial treatment of Sorafenib, a quizartinib (AC220) and crenolanib, a FLT3-ITD inhibitors | AML | Lethal mitophagy | Preclinical trials | Stein and Tallman (2016) |
| Combinatorial treatment of FH535, a synthetic inhibitor of the Wnt/β-catenin pathway; FH535-N, a derivative of FH535; and sorafenib | HCC cell line (Huh7, Hep3B, and PLC) | Wnt/β-catenin pathway | Preclinical trials | Turcios et al. (2019) |
| LCL-461, a mitochondria-targeted ceramide analog drug | FLT3-ITD + AML | Lethal mitophagy | Preclinical trials | Dany et al. (2016) |

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| CerS1/C18-ceramide, a central molecule of sphingolipid metabolism | Squamous head and neck cancer cell lines (UM-SCC-22A and UM-SCC-22B) | Lethal mitophagy | Preclinical trials | Sentelle et al. (2012) |
| Doxorubicin, a DNA damaging agent | Colorectal cancer stem cells (HCT8) | Inhibition of mitophagy by *BNIP3L* silencing | Preclinical trials | Yan et al. (2017) |
| Salinomycin, an antibacterial and coccidiostat ionophore drug | Prostate cancer cell line (PC3), breast cancer cell lines (SKBR3 and MDA-MB468) | Mitochondrial hyperpolarization | Preclinical trials | Jangamreddy et al. (2013) |
| UNBS1450, a sodium channel antagonist | Stromal neuroblastoma SH-N-AS cell line | Inhibition of autophagy by small inhibitory RNAs targeting Atg5, autophagy related 7 (Atg7), and Beclin-1 | Preclinical trials | Radogna et al. (2016) |
| Combinatorial treatment of temozolomide, a DNA alkylating agent and ganciclovir, a synthetic guanine derivative | Glioblastoma multiforme | Arrested tumor growth | Preclinical trials | Chen et al. (2012) |
| Combinatorial treatment of photosan-II (PS-II)-mediated photodynamic therapy (PS-PDT) and autophagy inhibitors | Colorectal cancer cell lines (SW620 and HCT116) | Downregulated AKT-mTOR pathway | Preclinical trials | Xiong et al. (2017) |
| Rapamycin, an inhibitor of the mammalian target of rapamycin (mTOR) | Pulmonary diseases | Inhibition of mTOR | Clinical trials: NCT01462006 | Galluzzi et al. (2017) |
| Temsirolimus, a rapamycin analog | Renal cell carcinoma | Inhibition of mTOR | Clinical trials: NCT01404104 | Kwitkowski et al. (2010) |
| Everolimus, a rapamycin analog | Pancreatic NET | Inhibition of mTOR | Clinical trials: NCT02305810 | Yao et al. (2013) |

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**Table 10.2** (continued)

| Anticancer therapy | Type of cancer | Molecular pathway targeted | Current status | References |
|---|---|---|---|---|
| Combinatorial treatment of SAR405, a kinase inhibitor of Vps18 and Vps34, and everolimus | Renal cancer cell lines (ACHN and 786-O) | Impairs lysosomal function inhibition of cancer proliferation | Preclinical trials | Ronan et al. (2014) |
| Chloroquine or hydroxychloroquine, an aminoquinoline-a late-stage autophagy inhibitor | Bladder cancer cell lines (5637 and T24) and pancreatic adenocarcinoma cell lines MiaPaCa2 (nonmetastatic) and S2VP10 (metastatic) | Targeting basal autophagy | Preclinical trials | Frieboes et al. (2014), Lin et al. (2017) |
| Combinatorial treatment of temsirolimus and hydroxychloroquine | Advanced solid tumors and melanoma | Temsirolimus-associated induction of autophagy and hydroxychloroquine-associated block in the clearance of autophagic vacuoles | Clinical: NCT00909831 + preclinical trials | Rangwala et al. (2014) |
| Combinatorial treatment of Lys05, a water-soluble analog of hydroxychloroquine and PLX4720, a BRaf kinase inhibitor | Melanoma (MEL624) | Lysosomal autophagy inhibition | Preclinical trials | Ma et al. (2014) |
| Quinacrine (DQ661), derived from Lys05 | Melanoma, colon cancer, and breast cancer | Inhibition of PPT1 causing mTOR inhibition | Preclinical trials | Rebecca et al. (2019) |
| Combinatorial treatment of vemurafenib, an inhibitor of the BRaf enzyme, and hydroxychloroquine | Metastatic BRaf V600E + melanoma | Inhibition of PERK arm of the ER stress response | Clinical trials: NCT01897116 | Ma et al. (2014) |
| Combinatorial treatment of chloroquine or hydroxychloroquine and gemcitabine, an antimetabolite | Pancreatic cancer stem cells (primary PDAC tumors) | Inhibition of CXCL12/CXCR4 signaling | Clinical: NCT01777477, NCT01128296 + preclinical trials | Balic et al. (2014) |

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|---|---|---|---|---|
| Combinatorial treatment of chloroquine or bafilomycin A1, another late-stage autophagy inhibitor with IM and nilotinib, or dasatinib, a tyrosine kinase inhibitor | Chronic myeloid leukemia (K562, BV173, IM-treated 32D-p210$^{BCR/ABL}$) | Inhibition of autophagy | Preclinical trials | Bellodi et al. (2009) |
| Combinatorial treatment of Bevacizumab, a blocker of EGFR, or Temozolomide, with chloroquine | Glioblastoma stem cells (U87-MG) | Suppressing Akt/mTOR signaling | Preclinical trials | Huang et al. (2018) |
| Triple combinatorial treatment of 5-fluorouracil, chloroquine, and Notch inhibitor | Gastric CSCs (MGC-803 and MKN-45) | Inhibition of Notch signaling pathway | Preclinical trials | Li et al. (2018) |
| Triple combinatorial treatment of HCQ, radiation therapy (RT), and temozolomide (TMZ) | Glioblastoma (GB) | Autophagy inhibition | Clinical trials: NCT00486603 | Rosenfeld et al. (2014) |
| Triple combinatorial treatment of chloroquine, PI3K/Akt pathway inhibitor along with gamma-irradiation | Primary stem-like glioma cells. | Inhibition of late autophagy | Clinical + preclinical trials | Firat et al. (2012) |
| Spautin-1, a novel autophagy inhibitor | Imatinib mesylate (IM)-resistant chronic myeloid leukemia (CML) | Inhibition of PI3K/AKT | Preclinical trials | Shao et al. (2014) |
| Combinatorial treatment of Dofequidar Fumarate, an inhibitor of ABC transporters and Docetaxel | Chronic myeloid leukemia (K562), breast cancer (BSY-1, HBC-4, and HBC-5), glioma human (U251), pancreatic cancer (Capan-1), colon cancer (KM12), and stomach cancer (MKN74) | Inhibit the efflux of chemotherapeutic drugs and increase the sensitivity to anticancer drugs in CSCs | Clinical: NCT00004886 + preclinical trials | Katayama et al. (2009) |

(continued)

**Table 10.2** (continued)

| Anticancer therapy | Type of cancer | Molecular pathway targeted | Current status | References |
|---|---|---|---|---|
| Combinatorial treatment of Demcizumab, humanized monoclonal antibody with Pembrolizumab, a humanized antibody used in cancer immunotherapy | Metastatic solid tumors | Inhibits Delta-like ligand 4 (DLL4) in the Notch signaling pathway, target CSCs, bulk tumor, and angiogenesis | Clinical trials: NCT02722954 | Previs et al. (2015) |

Park et al. 2013). Additionally, the CXCL12/CXCR4 axis is prompted in colorectal cancer and is linked with potential progression of cancer, such as invasion, metastasis, and chemoresistance. Subsequently, grants 5-fluorouracil (5-FU) resistance by increasing autophagy *both* in vitro and in vivo (Yu et al. 2017).

Suppression of autophagy preferentially stimulated in multiple molecular pathways that govern CSCs growth and differentiation, includes Notch (Li et al. 2018), Sonic Hedgehog (Fan et al. 2019), Wnt/β-catenin (Pai et al. 2017), NF-kβ (Trocoli and Djavaheri-Mergny 2011), transforming growth factor-β (Kiyono et al. 2009), and fibroblast growth factor (Chen et al. 2018) signaling cascades lead to sensitization of cancer cells to anticancer therapy. The appreciating effect of the Wnt/β-catenin pathway is inhibited by FH535 and its derivative (FH535-N) alone and in combination with sorafenib through nullification of the autophagic flux in hepatocellular carcinoma (Turcios et al. 2019). Hyperactivation of PI3K/Akt/mTOR pathway in GBM and its inhibition exerts antineoplastic activity by targeting CSCs, supporting differentiation, and inhibiting cell migration and invasion prospective of GSCs (Li et al. 2016). Balance is the key between Beclin1 and Bcl2/Bcl-xL that supports the concept of the presence of a complex relationship between autophagy and apoptosis, which seems important in the context of cancer and cancer therapy (Kim et al. 2014). JNK-mediated protective autophagy increased Bcl2 expression followed by an increased autophagic flux and conferred chemoresistance in colon cancer (Sui et al. 2014).

Evolving clinical and experimental evidence indicates that CSCs have clinical significance as they are bestowed with intrinsic resistance to radio- and chemotherapy owing to the indulgence of autophagy (Chen et al. 2012; Vitale et al. 2015). Targeting components of the autophagic machinery can be recruited as the hopeful target to selectively eliminate CSCs facilitating cancer cell growth/progression/metastasis and enhancing the effectiveness of radio- and chemotherapy (Nazio et al. 2019; Ojha et al. 2015; Perez-Hernandez et al. 2019). Henceforth, these findings completely indicate that autophagy suppression and its activation, both, can be deemed to be promising approaches for sensitizing CSCs to anticancer therapy, evaluated by the reduction of the number of CSCs. So, the development of new anticancer drugs focuses on CSCs which is key to the problem required to be resolved in drug clinical trials (Fig. 10.2).

## 10.4 Conclusion

Development of autophagy inhibitors, specific mitophagy inhibitors have been proven beneficial, given the fears about global autophagy suppression for tissue homeostasis and that mitophagy has a crucial functional role earlier credited to general autophagy. Focusing on selective inhibitors will pave an unexplored path of how autophagy is responsible for determining stemness, dormancy-whether DTCs are autophagy-dependent CSCs, and which autophagy functions will be significant in promoting drug resistance and cancer recurrence. Further research is requisite before CSCs can be treated by regulating autophagy and mitophagy.

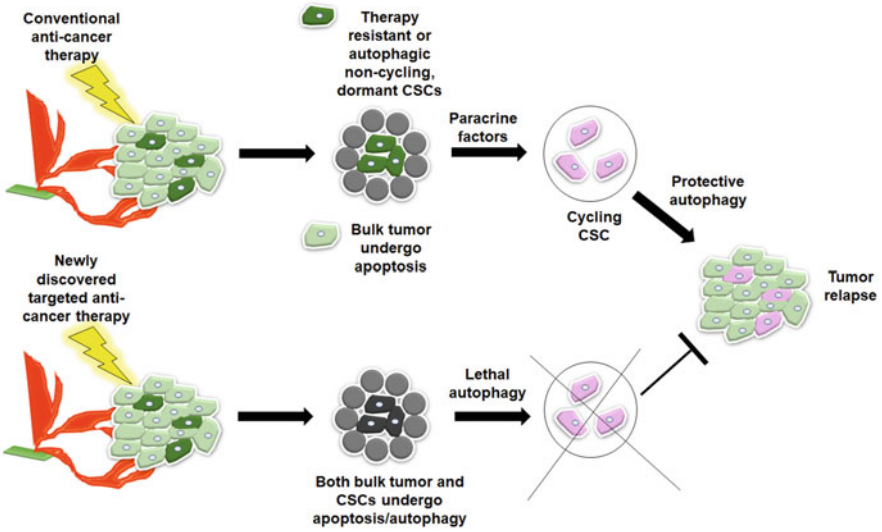


**Fig. 10.2** The conventional anticancer therapy is incapable to target the CSCs that shoot to cancer relapse. Autophagy plays a Janus role in cancer cell modulation, acts protective during tumor relapse, and lethal via programmed cell death. Newly discovered combinatorial treatments target both the bulk tumor and cancer stem cells leading to elimination of persistent CSCs and tumor regression

Desirable therapeutic impacts of anticancer reagents have not been achieved by only targeting autophagy using autophagy modulators; to the contrary, it has enacted as a pro-survival response by supplying nutrients to cancer cells. Consequently, clinical trials that aim autophagy by a combination of autophagy alterations and anticancer components are appropriate to consider autophagy as a possible effectual therapeutic approach in anticancer therapy. The conjunction of these techniques hopefully deciphers the vital mechanisms necessary for maintaining cancer stemness and will play an important role in designing more efficient and effective personalized therapeutic strategies.

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