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Review

The role of autophagy in squamous cell carcinoma of the head and neck

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SUMMARY

Half a million new head and neck cancers are diagnosed each year worldwide. Although traditionally thought to be triggered by alcohol and smoking abuse, there is a growing subset of oropharyngeal cancers driven by the oncogenic human papilloma virus (HPV). Despite advances in both surgical and non-surgical treatment strategies, survival rates have remained relatively static emphasising the need for novel therapeutic approaches. Autophagy, the principal catabolic process for the lysosomal – mediated breakdown of cellular products is a hot topic in cancer medicine. Increasing evidence points towards the prognostic significance of autophagy biomarkers in solid tumours as well as strategies through which to harness autophagy modulation to promote tumour cell death. However, the role of autophagy in head and neck cancers is less well defined. In the present review, we summarise the current understanding of autophagy in head and neck cancers, revealing key areas for future translational research.

Introduction

Head and neck tumours are responsible for around half a million new cancers worldwide each year [1]. Although these heterogeneous tumours are traditionally thought to be driven by excessive alcohol and cigarette consumption [2], there are a growing number of cancers, mainly in the oropharynx, driven by the oncogenic human papilloma virus (HPV) [3]. Significant advances in surgical [4], radiological [5] and chemotherapeutic [6] strategies have led to reduced morbidity. However, improvements in 5 year survival rates are only apparent in a few sub-sites of head and neck cancers [7] in part reflected by a change in disease demographics; patients with HPV positive oropharyngeal cancers have higher overall and progression free survival compared to their negative counterparts [8]. Consequently, there is a clear need for novel prognostic biomarkers and targeted treatment approaches to head and neck disease.

Macro-autophagy (referred to from here as 'autophagy') is the principle intracellular, lysosomal mediated, evolutionarily conserved process in all eukaryotes for the breakdown and recycling of damaged organelles or excess proteins [9]. Activated in times of nutrient deprivation or stress to sustain cell survival [10], autophagy comprises of 5 phases (Fig. 1) and is controlled by a family of autophagy or Atg related regulatory proteins and the interaction of key protein kinase complexes [11].

The mammalian target of rapamycin (mTOR) essentially serves as a central regulator of autophagy. Activated in times of nutrient availability, mTOR maintains basal levels of autophagy (13). Inactivation in times of nutrient deprivation or cellular stress triggers the formation of an isolation membrane from cellular apparatus including the endoplasmic reticulum [12] and the sequestration of cytoplasmic material to be degraded into a double membraned autophagosome [9]. Subsequent fusion of the autophagosome with a lysosome then results in the formation of an autolysosome where cytosolic components are broken-down to free building blocks that are later recycled to sustain cellular homeostasis [9]. This process is mediated by a family of autophagy regulatory genes (Atg) with induction regulated by 2 key protein kinase complexes, the ULK1/2 complex and a class III PI3 kinase complex containing vps34, beclin-1 (ATG-6) and Ambra-1 (Fig. 1) [13]. These complexes, in concert with 2 key conjugation systems (Atg12/Atg5/Atg16 and microtubule associated protein 1 light chain 3 (LC3 – a protein conjugated to phosphatidylethanolamine (PE) to form LC3-II upon autophagy induction)) maintain correct autophagic flux (Fig. 1).

The complete process of autophagy or autophagic flux can be monitored *in vitro* at the ultra-structural level by electron microscopy to detect double membraned autophagosomes [14] and by LC3-lipidation [15,16]. In tissue, the expression of beclin-1 [17,18], the degradation of SQSTM1/p62 [19] and endogenous LC3-II [20] have all been described as autophagy biomarkers.

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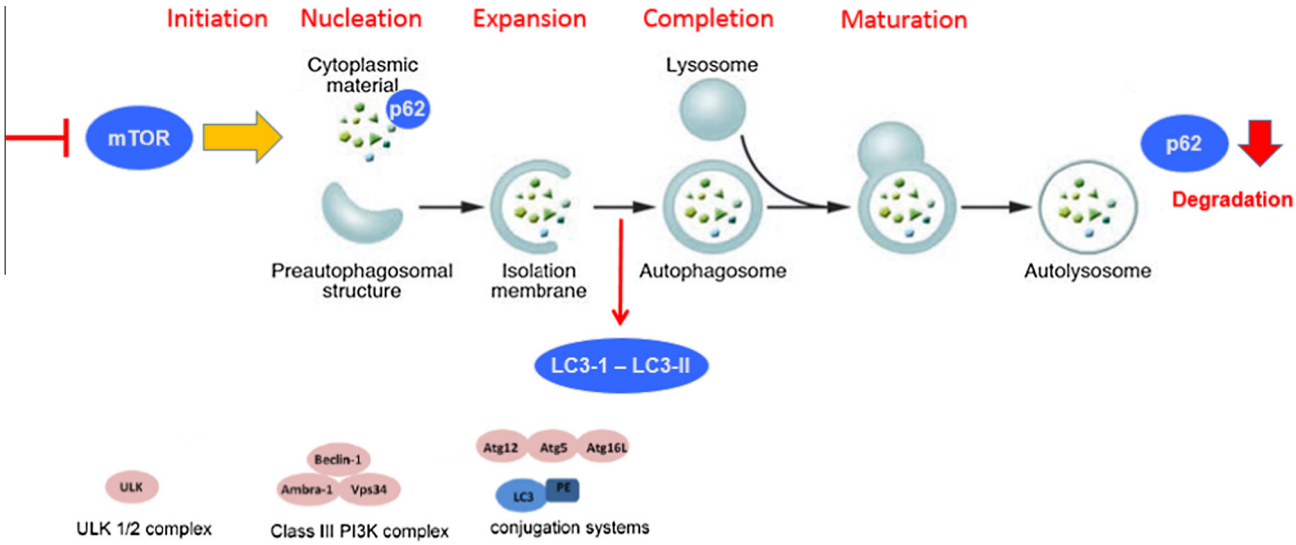

Fig. 1. Process of autophagy with selected regulatory proteins.

In the context of cancer, several studies have also described the successful modulation of autophagy for therapeutic benefit. For example, inhibition of autophagy with lysosomal inhibitors such as hyrdroxychloroquine in combination with anti-cancer drugs such as temozolomide promotes cell death *in vitro* [21] with early stage clinical studies in melanoma suggesting anti-tumour effects *in vivo* [22].

However, there is some evidence from early clinical trials that autophagy inhibition with hydroxychloroquine may cause side effects that limit its clinical application. This includes the theoretical risk of secondary tumour development and the possible potentiation of serious complications including acute kidney injury [23]. It has therefore been postulated that clinically achievable concentrations may not be effective at inhibiting autophagy *in vivo* [24]. However, hydroxychloroquine is not a specific autophagy inhibitor and as more specific inhibitors come onto market, such as those to vps34 [25], their efficacy and side effect profile may improve. Nevertheless, there are other ways to modulate autophagy to decrease tumour cell viability. For example, the use of cannabinoid derivatives to exacerbate autophagy and promote cell death are currently showing promise as an adjuvant therapy for glioma [26] and melanoma [27].

Whilst the potential for key regulatory proteins to act as prognostic biomarkers has been described in many cancer types, few studies to date have explored the role of this key cellular signalling mechanism or strategies through which it may be harnessed for the therapeutic benefit of head and neck cancers. This review thus aims to summarise what is currently known about autophagy in squamous cell cancers of the head and neck and identify important areas for future translational research.

Discussion

Oral cavity

Of all the head and neck cancer subsites, autophagy has been investigated most frequently in the oral cavity. A recent immunohistochemical study of 195 oral squamous cell cancers revealed higher levels of cytoplasmic p62, suggesting impaired autophagy, and its correlation with reduced overall and disease specific survival [28]. Interestingly these authors also observed increased levels of LC3-II in patients with poor outcome further suggesting increased basal levels of autophagy in these tumours may be linked to autophagy re-activation in advanced stage cancers. This has been shown in other cancers such as melanoma [19] and supports the notion of an autophagy paradox where autophagy dysregulation in early stage cancers promotes tumourigenesis whereas it may be reactivated as an attempt to promote survival in later stage disease [29]. Further studies of p62 in a smaller report of 54 tumours by Inui et al. [30] also described its increased expression and its association with reduced disease specific survival.

Endogenous LC3-II expression has been reported in 90 oral cavity tumours [31] revealing the association of 'high' levels of LC3-II with reduced overall survival. This again supports the notion of autophagy reactivation during disease progression. These observations have also been reported in a further study of 74 oral cavity tumours in which LC3-II expression in the tumour periphery was also associated with reduced overall survival [32]. However, it is important to note that autophagy is a dynamic process and that lysosomal turnover, rather than expression of LC3-II at one moment in time, is a true marker of a tumour's autophagic state [33]. As such, the expression of LC3-II in formalin fixed, paraffin embedded tissue may not be the most appropriate way to measure autophagy in this setting.

Autophagy in oral cancer cell lines has also been studied in the context of regulatory gene expression. Investigations by Wu et al. [34] demonstrated that ionizing radiation induces both autophagy and cellular death. However, it is not clear whether cell death was the result of cytotoxic autophagy or whether autophagy was activated as a result of radiation induced cellular stress. Furthermore, it is clear that in some human oral cancer cell lines, autophagy modulation results in an altered tumour phenotype; knockdown of Beclin-1 apparently promotes the proliferation, migration and invasion of primary oral cancer cell lines suggesting that the inhibition of autophagy may promote a more aggressive cancer phenotype [35]. Interestingly, the same group later reported observations of reduced beclin-1 activity in a cohort of oral cavity tumours and the correlation with a more advanced disease stage [36].

A number of other studies have investigated the effect of autophagy modulation on cell death in oral cancer cell lines. A study of the concomitant treatment of chemoresistent oral cancer cell lines with cisplatin and the autophagy inhibitors chloroquine and 3-methyladenine (3-MA) demonstrated increased apoptosis [37] suggesting autophagy inhibition may be a useful adjuvant strategy for patients receiving chemotherapy. However, despite cisplatin