Is LAMB3 a Therapeutic Target for Squamous-Cell Carcinoma?

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Squamous-cell carcinoma (SCC) is the most common cancer capable of metastasis with high morbidity and mortality, and can cause head and neck, lung, gastric and papillary thyroid cancers, among others. However, existing therapies show limited effects for patients. Laminin subunit beta-3 (LAMB3), which encodes one of the three subunits of Laminin-332, plays a crucial role in tumorigenesis and progression of several types of cancer. This review aims to examine the evidence for a role of LAMB3 in SCC as well as its potential to be a therapeutic target for SCC, and then puts forward several possible anticancer therapies using LAMB3 as target. It was reported that LAMB3 is upregulated in tumour tissues, which is associated with lymph metastasis. LAMB3 could promote cell metastasis ability by regulating EMT-related proteins, MMPs and PI3K/Akt signalling pathway, and thus contributing to tumour progression. LAMB3 could also promote cell proliferation by altering the cell cycle distribution and affecting apoptosis in some cases but there also exist some cases where LAMB3 had no effect on cell proliferation, which requires further investigation. Therefore, LAMB3 is a promising diagnostic marker as well as a potential therapeutic target for SCC. Regulating LAMB3 by targeting at relative regulatory miRNAs, inducing epigenetic modification such as DNA or histone methylation, and CRISPR/dCas9 editing tool would all be possible anticancer therapies. Henceforth, targeting LAMB3 could provide improved therapies for SCC patients with a better clinical response.

Introduction

Squamous-cell carcinoma (SCC) is a highly prevalent invasive malignant neoplasm with high morbidity and mortality. SCC, which is recognized as the most common cancer capable of metastasis, could arise in various tissues and give rise to a wide range of cancers (Marinkovich, 2007). SCC tumours can often invade adjacent tissues and metastasize to distant sites such as lymph nodes (Tran *et al.,* 2008). So far, chemotherapy for SCC has shown limited effects for patients and the mortality rate of SCC is still high despite existing therapies (Marinkovich, 2007). Hence, searching for an efficient therapeutic target for treating SCC is necessary and beneficial.

Laminins, which are crucial components of basement membrane zones, are large extracellular glycoproteins associated with a variety of biological processes including cell migration, adhesion, proliferation, and interactions with other extracellular matrix components (Patarroyo *et al.,* 2002; Jung *et al.,* 2018). Laminin-332 (formerly termed laminin-5), one of the laminin isoforms, consists of three subunits, α3, β3 and γ2, which are coded by LAMA3, LAMB3 and LAMC2 genes respectively (Benati *et al.,* 2018; Fortugno *et al.,* 2020) (Figure 1). Laminin subunit beta-3 (LAMB3) plays a crucial role in tumorigenesis and progression of several types of cancer such as head and neck squamous cell carcinoma (HNSCC), pancreatic ductal adenocarcinoma (PDAC), papillary thyroid cancer (PTC), gastric cancer and lung cancer (Liu *et al.,* 2019; Zhang *et al.,* 2019; Jung *et al.,* 2018; Huang *et al.,* 2020; Wang *et al.,* 2017; Kwon *et al.,* 2011; Wang *et al.,* 2013). This review aims to examine the evidence for a role of LAMB3 in SCC to determine if it holds potential as a therapeutic target.

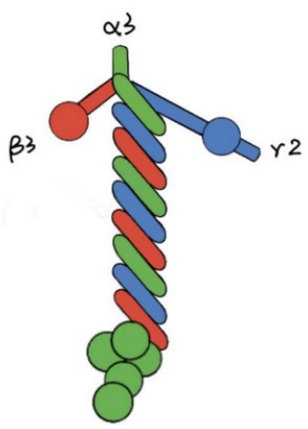

Figure 1. Laminin-332 structure with the β3 subunits in red.

LAMB3 is Positively Correlated with Tumorigenesis

Many studies performed experiments to determine the LAMB3 expression in SCC and they all showed the higher LAMB3 expression in tumour tissues than that in unaffected tissues at both mRNA and protein levels (Liu *et al.,* 2019; Zhang *et al.,* 2019; Jung *et al.,* 2018; Huang *et al.,* 2020; Kwon *et al.,* 2011; Wang *et al.,* 2013). Expression levels of LAMB3 can be used to differentiate tumour tissues and unaffected ones (Wang *et al.,* 2017) and thus it could be recognized as a promising biomarker for SCC. Through analysing the association of LAMB3 expression and clinicopathological features, higher LAMB3 expression is associated with increasing risk of lymph nodes metastasis (Liu *et al.,* 2019; Wang *et al.,* 2017; Wang *et al.,* 2013). Lymph nodes metastasis is highly correlated to the poor prognosis including local recurrence and cancer-specific mortality preoperatively (Guo *et al.,* 2020; Xing *et al.,* 2020). This poor prognosis, which is also correlated with the overexpression of LAMB3, involves many types of cancer such as NHSCC, PDAC and PTC (Liu *et al.,* 2019; Zhang *et al.,* 2019; Jung *et al.,* 2018).

Taken together, LAMB3 could be a prognostic biomarker for certain cancer types. Therefore, this promoted the question about whether LAMB3 contributes to tumour progression.

Controversy on the Effect LAMB3 Has on Cell Proliferation

Previously it was found that LAMB3 could promote cell proliferation in PDAC, PTC and gastric cancer cells (Kwon *et al.,* 2011; Huang *et al.,* 2020; Zhang *et al.,* 2019; Wang *et al.,* 2017). In these cases, among cells with higher expression of LAMB3 compared with unaffected cells, a larger proportion of them would be in G2/M phase while a smaller proportion would be in G1 phase. LAMB3 overexpression could lead to the upregulation of both cyclin D and BCL-2, and downregulation of p53, while its knockdown can induce the opposite effect. Cyclin D could promote the progression of cell cycle, BCL-2 plays a role in inhibiting apoptosis, and p53 could induce cell cycle arrest and promote apoptosis (Zhang *et al.,* 2019). Hence, LAMB3 can promote cell proliferation by altering the cell cycle distribution and reducing the number of early apoptotic cells (Zhang *et al.,* 2019; Huang *et al.,* 2020). However, this is not universally the case, there have been some reports where LAMB3 had no significant effect on cell proliferation in HNSCC and PTC (Liu *et al.,* 2018; Jung *et al.,* 2018). Especially, two previous studies from 2017 and 2018 respectively held contrary conclusions on the effect that LAMB3 has on cell proliferation in PTC (Wang *et al.,* 2017; Jung *et al.,* 2018). Thus, LAMB3 may promote cell proliferation in some tumour subtypes but not in other subtypes. However, no certain conclusion can be drawn on it at present and more relative studies are required.

LAMB3 Contributes to Tumour Progression in Multiple Ways

It is commonly accepted that in several cancer types LAMB3 could promote cell invasion and migration, which are two key steps of metastasis (Liu *et al.,* 2019; Zhang *et al.,* 2019; Jung *et al.,* 2018; Wang *et al.,* 2017; Kwon *et al.,* 2011; Wang *et al.,* 2013). Metastasis is a crucial step in tumour progression and could give rise to related death and treatment failure (Bray *et al.,* 2018). One way that LAMB3 contributes to tumour progression is by regulating epithelial-to-mesenchymal transition (EMT)-related proteins expression (Liu *et al.,* 2019; Zhang *et al.,* 2019; Jung *et al.,* 2018; Fukazawa *et al.,* 2015). EMT is associated with cell invasion in tumour progression (Jung *et al.,* 2018). During the EMT, epithelial cells lose cell-cell adhesion and then are transited to mesenchymal cells, which allows the tumour cells to invade adjacent tissues and metastasize to distant sites (Fukazawa *et al.,* 2015). Knockdown of LAMB3 has been shown to induce the upregulation of E-cadherin and downregulation of N-cadherin, vimentin and Slug (Zhang *et al.,* 2019; Liu *et al.,* 2018), where E-cadherin, N-cadherin, Slug and vimentin are well-known EMT-related proteins (Jung *et al.,* 2018). The opposite effect is observed when LAMB3 is upregulated, indicating the role of LAMB3 in regulating EMT-related proteins expression.

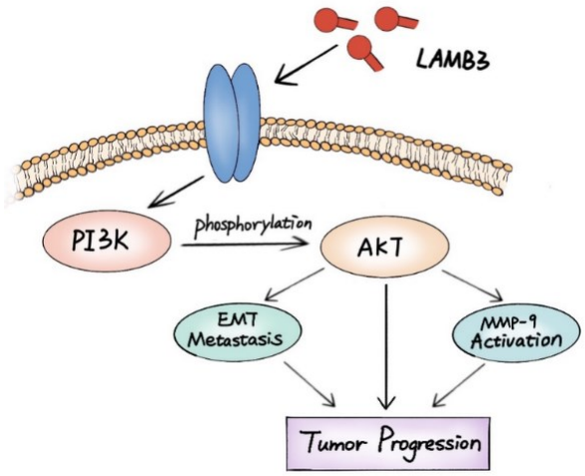

Figure 2. Mechanisms underlying the PI3K/Akt signalling pathway.

Regulating the matrix metalloproteinases (MMP) could also contribute to tumour progression by LAMB3 (Zhang *et al.,* 2019; Jung *et al.,* 2018; Kwon *et al.,* 2011; Wang *et al.,* 2013; Udayakumar *et al.,* 2003; Remy *et al.,* 2006). In order to invade the adjacent tissues and migrate to distant sites, the cancer cells have to degrade the basement membrane (BM) which acts like a barrier against cell metastasis. Normally this degradation of BM is processed via the MMP family including MT1-MMP (MMP-14), MMP-7, MMP-2 and MMP-9 (Remy *et al.,* 2006). MT1-MMP (MMP-14), which is important for the initiation of BM breakdown, is also found to activate MMP-2, which is another metastasis-related protein. LAMB3 chain is a target for MT1-MMP to cleave, thus promoting cell metastasis (Udayakumar *et al.,* 2003). Likewise, LAMB3 chain acts as a specific ligand for MMP-7, facilitating the cleavage processed by MMP-7 and enhancing cell metastasis. MMP-7, which is mainly expressed in cells of carcinomas, plays an important role in BM degradation and is associated with distant and lymph node metastasis (Remy *et al.,* 2006). In 2018, a study on PTC reported that pro-MMP-9 mRNA expression and pro-MMP-9 secretion were significantly inhibited by LAMB3 knockdown where MMP-9 is integral to cell metastasis (Jung *et al.,* 2018). Taken together, LAMB3 contributes to tumour progression by associating with MMPs.

Moreover, LAMB3 could also regulate the PI3K/Akt signalling pathway to promote tumour progression (Zhang *et al.,* 2019; Jung *et al.,* 2018; Huang *et al.,* 2020; Wang *et al.,* 2017). This pathway involves phosphatidylinositol 3-kinase (PI3K) and protein kinase B (PKB/Akt), having a fundamental role in tumour progression. The activation of PI3K can mediate the phosphorylation of Akt, regulating cell invasion and migration (Xu *et al.,* 2018), The knockdown of LAMB3 could inhibit the transcription and activation of PI3K, leading to significant reduction in Akt phosphorylation, and thus giving rise to the suppression of cell invasion and migration (Zhang *et al.,* 2019). Hence, LAMB3 promotes the tumour progression by activating the PI3K-mediated Akt phosphorylation. This activation of the pathway also induces EMT and activates MMP-9, contributing to cell metastasis (Jung *et al.,* 2018) (Figure 2).

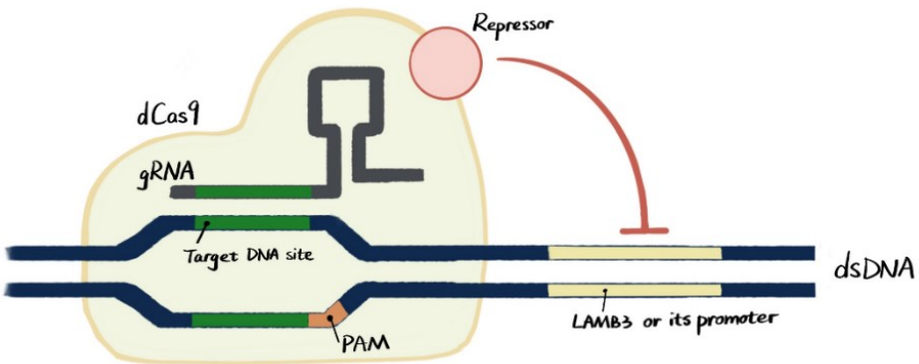

Figure 3. The mechanism of CRISPR/dCas9 gene editing tool.

Is LAMB3 a Potential Therapeutic Target for SCC?

LAMB3 was reported to be a potential diagnostic marker (Wang *et al.,* 2017), and several studies had mentioned about regulating LAMB3 as a novel therapeutic strategy (Huang *et al.,* 2020; Kwon *et al.,* 2011; Benati *et al.,* 2018), representing its potential to be a therapeutic target for SCC. One possible way is regulating the expression of LAMB3 by targeting the relative regulatory miRNAs. As most mammalian mRNAs can be conserved targets of miRNAs, finding the miRNA that could regulate LAMB3 wound be a possible approach (Friedman *et al.,* 2009). Huang *et al.,* (2020) found miR-24-3p that is an upstream miRNA of LAMB3 and could directly target the 3'UTR of the mRNA of LAMB3. And it was further shown that the miR-24-3p/LAMB3 axis could be a target for an anticancer therapeutic strategy (Huang *et al.,* 2020). Likewise, there may exist other relative miRNA could target and regulate the LAMB3 expression.

Histone methylation and DNA methylation could be another way to regulate the expression of LAMB3 given that epigenetic modification is a crucial mechanism for regulating gene expression. Kwon *et al.,* (2011) reported a strong correlation between LAMB3 expression and DNA methylation as well as histone methylation. Both promoter demethylaion and active H3K4me3 mark results in upregulation of LAMB3 (Kwon *et al.,* 2011). This previous study showed the possibility of regulating the LAMB3 expression by epigenetic mechanism. Thus, inducing the hypermethylation of LAMB3 gene promoter could be a possible way to downregulate LAMB3 expression.

LAMB3 could also be regulated by CRISPR system. CRISPR systems, which have been applied successfully in mammalian cells, can not only conduct the genome engineering with Cas9 nuclease, but also localize the target gene with dead Cas9 (dCas9) nuclease (Xiong *et al.,* 2020; Moses *et al.,* 2020). The dCas9 can be fused with the repressor domain and directed to the target DNA site guided by gRNA, thus repressing the gene expression (Moses *et al.,* 2020) (Figure 3). Likewise, dCas9 may be fused with protein that is associated with epigenetic modification, regulating LAMB3 expression via epigenetic modification such as histone methylation.

Conclusion

LAMB3 is upregulated in tumour tissues, which is associated with lymph metastasis. However, its role in cell proliferation requires further investigation as it could alter the cell cycle distribution and reduce the number of early apoptotic cells in some cases but not in other cases. LAMB3 could also promote cell invasion and migration ability and thus contribute to tumour progression by regulating EMT-related proteins, MMPs and PI3K/Akt signaling pathway. Therefore, LAMB3 is a promising diagnostic marker as well as a potential therapeutic target for SCC. Regulating LAMB3 by targeting at relative regulatory miRNAs, inducing epigenetic modification such as DNA or histone methylation, and CRISPR/dCas9 editing tool would all be possible anticancer therapies. Hence, LAMB3 is a potential therapeutic target for SCC and targeting it could provide improved therapies for SCC patients with a better clinical response.

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