The ITGB6 gene: its role in experimental and clinical biology

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ABSTRACT

Integrin αvβ6 is a membrane-spanning heterodimeric glycoprotein involved in wound healing and the pathogenesis of diseases including fibrosis and cancer. Therefore, it is of great clinical interest for us to understand the molecular mechanisms of its biology. As the limiting binding partner in the heterodimer, the β6 subunit controls αvβ6 expression and availability. Here we describe our understanding of the ITGB6 gene encoding the β6 subunit, including its structure, transcriptional and post-transcriptional regulation, the biological effects observed in ITGB6 deficient mice and clinical cases of ITGB6 mutations.

Introduction

Integrins are heterodimeric cell surface proteins that facilitate signalling between the intracellular and extracellular environments. Each integrin consists of one α and one β subunit. Together, they are capable of forming 24 unique integrins in humans (reviewed by Hynes, 2002). αvβ6 is expressed exclusively on epithelial cells but is absent or expressed at low levels in normal healthy adult tissue (Breuss et al., 1993). In contrast, αvβ6 expression is increased during development (Breuss et al., 1995), wound healing (Haapasalmi et al., 1996), cancer (reviewed by Bandyopadhyay and Raghavan, 2009) and fibrosis (Munger et al., 1999), all of which are processes requiring tissue remodelling (Fig. 1).

αvβ6 binds to proteins containing the RGD (Arg-Gly-Asp) motif (Buck et al., 1992). Well characterised αvβ6 ligands include fibronectin (Buck et al., 1992) and the Transforming Growth Factor Beta (TGF-β) pro-peptide, Latency Associated Peptide (LAP) (Munger et al., 1999; Annes et al., 2002). LAP is post-translationally cleaved from TGF-β but remains directly associated with the cytokine, anchored as a homodimer to the extracellular matrix (ECM) via latent TGFβ binding protein 1 (Annes et al., 2004). This whole complex remains inert and is referred to as latent TGF-β1. Binding of αvβ6 to LAP physically moves LAP and exposes TGF-β1 to its receptors on adjacent cells, permitting an actomyosin dependent activation of TGF-β1 (Munger et al., 1999, Annes et al., 2004). Binding domains are also present on the cytoplasmic tail of integrin β6, which recruits signalling and structural partners including HAX-1, Fyn, ERK2, and Kindlin-1 (Ahmed et al., 2002; Li et al., 2003; Ramsay et al., 2007; Moser et al., 2008). Extracellular ligand binding to αvβ6 is able to effect a conformational change in the cytoplasmic tail and thus initiate downstream signalling (outside-in signalling), while cytoplasmic tail binding may also affect extracellular ligand binding (inside-out signalling).

Through these and other signalling pathways, αvβ6 regulates many basic functions of the cell, such as proliferation (Agrez et al., 1994), migration (Huang et al., 1998), and ECM degradation (Thomas et al., 2003; Ramsay et al., 2007; Moser et al., 2008). Extracellular ligand binding to αvβ6 is able to effect a conformational change in the cytoplasmic tail and thus initiate downstream signalling (outside-in signalling), while cytoplasmic tail binding may also affect extracellular ligand binding (inside-out signalling).

The β6 subunit was first isolated from guinea pig epithelial cells in 1990 (Sheppard et al., 1990). At this time, only three β subunit structures had been identified in mammalian cells, with emerging evidence for several other subunits (Cheresh et al., 1989). Sheppard and colleagues, observing that β1, β2 and β3 had large consensus regions in their sequences, designed primers against these conserved regions and successfully amplified β6. Further screening in human cell lines led to the identification of the β6 nucleotide sequence from the pancreatic cell line FG-2. It was later determined that ITGB6 was located on chromosome 2 (Krissansen et al., 1992), specifically 2q24.2 (Fernandez-Ruiz and Sanchez-Madrid, 1994).

Abbreviations: LAP, Latency Associated Peptide; TGFβ1, Transforming growth factor β1; OSCC, oral squamous cell carcinoma cells; STAT3, signal transducer and activator transcription 3; HDAC, Histone deacetylase; CREB, cAMP response element; HAT, histone acetyltransferase; UTR, Untranslated region

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Transcriptional regulation

As the exclusive binding partner of αv, β6 availability determines the cellular expression of αvβ6. Breakthrough work characterising the structure and transcriptional regulation of ITGB6 was performed by Xu et al., 2015. They identified both the transcriptional start site (TSS), 204 bp upstream of the translation initiation site, and the core promoter of ITGB6 using oral squamous cell carcinoma cells (OSCCs). A functional TATA box was located -24bp to -18bp upstream of the TSS, and binding sites for transcription factors C/EBPα and signal transducer and activator transcription 3 (STAT3) between -289bp and -150bp. Further binding sequences for Ets-1 and Smad proteins were also identified, however the basal rate of ITGB6 expression in OSCC cells was primarily defined by STAT3 and C/EBPα binding (Xu et al., 2015).

This was not the first time STAT3 had been implicated in the possible regulation of ITGB6. Previous work showed that constitutive STAT3 activation led to an increase in cell migration and motility in prostate cancer cell lines (Azare et al., 2007). This was attributed to an increase in ITGB6 mRNA expression within 4 hours of STAT3 simulation, whereas inhibition of STAT3 by shRNA resulted in downregulation of ITGB6 mRNA.

Work performed in colon carcinoma cell lines by Bates and colleagues in 2005 also revealed a binding domain for Ets-1 in the ITGB6 promoter region. As an initiating factor of the epithelial-mesenchymal transition (EMT), it was hypothesised that Ets-1 could possibly play a role in ITGB6 expression. They demonstrated that Ets-1 was able to bind to the ITGB6 promoter region and transactivate the promoter, resulting in increased ITGB6 transcription (Xu et al., 2015).

In 1992, it was discovered that TGF-β1 works as part of a positive feedback loop promoting expression of αvβ6 (Sheppard et al., 1992). In an effort to determine the mechanisms by which TGF-β1 regulates ITGB6 expression using a pulmonary fibrosis model, Tatler and colleagues identified the location of a suppressor region in the ITGB6 gene (Tatler et al., 2016a,b). They found that deletion of the region -818bp to -738bp leads to a significant increase in ITGB6 expression. This confirmed work by Xu and colleagues, who also showed that deletion from -909bp to -421bp leads to maximum transcriptional activity (Xu et al., 2015).

Another transcription factor of the Ets family was also shown to have a role in regulating ITGB6 (Tatler et al., 2016a,b). Following in silico analysis, it was found that the ETS domain-containing protein Elk1 was able to bind to a site in the apparent suppressor region and reduce ITGB6 expression. Loss of Elk1 in vivo led to an increased basal expression ITGB6 mRNA (Tatler et al., 2016a,b).

Smad transcription factors mediate classical TGF-β1 signalling (Derynck et al., 1998). Disruption of a Smad binding site within the same suppressor region of ITGB6 (-818bp to -738bp) led to abrogation of ITGB6 transcription induced by TGF-β1 (Tatler et al., 2016a,b). Inhibition of Smad3 led to a decrease in ITGB6 transcription and, moreover, Smad3/- mice treated with TGF-β1 had reduced expression of αvβ6. TGF-β1-induced ITGB6 expression regulated by Smad proteins has been extensively documented, including in bile duct epithelial cells (BDECs) where Smad3 inhibition reduced TGF-β1 induced ITGB6 mRNA (Verrecchia and Mauviel, 2002; Schiffer et al., 2000). Conversely, Xu and colleagues (2015) concluded that Smad binding domain activity was not essential for basal expression in OSCCs.

Regulation of ITGB6 by TGF-β1 was further explored by Xu and colleagues (2017). They identified that the AP1 subunit JunB binds directly to the ITBG6 promoter region. Furthermore, they identified a role for histone H3 and H4 acetylation following treatment with TGF-β. Inhibition of Histone Deacetylase (HDAC) resulted in increased acetylation of H3 and H4, leading to an increased expression of ITGB6. Histone acetylation was initiated by recruitment of the CAMP response element binding protein (CBP), which acts as both a histone acetyltransferase (HAT) and a scaffold that promotes the recruitment of further components required for gene transcription (Korzus et al., 2004).

Given that transcription of ITGB6 is almost certainly a mechanism determining its availability at the cell surface, understanding its regulation could identify potential therapeutic targets in diseases where αvβ6 is overexpressed. Although recent work has greatly improved our understanding of the structure of the promoter region, and binding sites...
of transcription factors, extensive work still remains to fully characterise how these transcription factors regulate ITGB6 transcription (Fig. 2).

Post-Transcriptional Regulation

Following gene transcription, protein expression may also be regulated by multiple post-transcriptional processes. One of the most significant points of post-transcriptional regulation happens at the initiation of mRNA translation. Eukaryotic translation initiation factor 4 (eIF4E) binds to the 5' end of mRNA, initiating protein translation (Rhoads, 1988). eIF4E is known to selectively increase the expression of proteins with 'weak' mRNA (Graff and Zimmer, 2003). This refers to proteins with a long and/or complex 5' UTR (untranslated region), usually proteins expressed at low levels in healthy tissue, as opposed to 'strong' mRNAs, which have a short and simple 5'UTR and are usually housekeeping genes. eIF4E expression is known to be dysregulated in malignancy (Siddiqui and Sonenberg, 2015). De Benedetti and Graff (2004) reviewed the effect of eIF4E in malignancy, describing its ability to selectively upregulate translation of some pro-tumorigenic proteins (e.g. Vascular Endothelial Growth Factor (VEGF)), which tend to have a more complex 5'UTR and require eIF4E for translation.

ITGB6 mRNA has a long 5'-UTR and is GC rich, both of which are characteristics of ‘weak’ mRNA. Combined expression of eIF4E and ITGB6 can predict a poorer prognosis in colon cancer (Niu et al., 2014). Furthermore, siRNA-induced depletion of eIF4E significantly reduced protein expression of β6 (Enyu et al., 2015). Further investigation in to eIF4E and αvβ6 expression in other models has yet to be performed. Thus, while we now know a significant number of the transcriptional regulators of ITGB6, we have not yet linked these data to how ITGB6 is upregulated in disease, especially cancer.

Mouse Models

Itgb6 knockout mice have been used to study the downstream effects of αvβ6 and improve our understanding of its biology. The first characterisation of Itgb6 deficient mice was published in 1996, with surprising results (Huang et al., 1996). Given the role αvβ6 plays in wound healing in human tissue (Haapasalmi et al., 1996), it was hypothesised that Itgb6 knockout mice would exhibit wound healing abnormalities. The authors also postulated that Itgb6 knockout mice would display developmental abnormalities due to the involvement of αvβ6 in cell migration and proliferation (Agrez et al., 1994; Huang et al., 1998). However, the Itgb6 knockout mice developed normally, with no difference in their wound healing ability. Rather, they exhibited moderate but significant inflammation in the lungs and skin. In the same study, Itgb6 knockout mice did not develop fibrosis in response to bleomycin. This phenotype is remarkably similar to TGF-β1 deficient mice, and is attributable to TGF-β1 activation by αvβ6 (Munger et al., 1999). Interestingly, inflammation was only observed in the skin and airways of Itgb6 knockout mice, despite the potential for expression of αvβ6 in other epithelial tissues. The authors offered this was perhaps due to the housing conditions of the mice causing irritation in the skin and lungs. Neonatal Itgb6 knockout mice also developed a temporary baldness. Whether this was due to the subsequent discovery that αvβ6 plays a role in hair follicle regeneration remains to be determined (Xie et al., 2012).

The fibrosis phenotype of Itgb6 knockout mice was further explored in Itgb6/Thrombospondin 1 (Tsp-1) double-null mice (Ludlow et al., 2005). Thrombospondin-1 also activates latent-TGF-β1 (Crawford et al., 1998) and in the double knockout model there was a higher incidence of inflammation and pneumonia, which more closely resembles the phenotype of TGF-β1 null mice (Shull et al., 1992). Whilst no significant differences in tumour incidence were observed between the Itgb6-/- and the Itgb6-/-; Tsp-1-/- mice, the authors found that Itgb6-/- mice developed both benign and malignant tumours at a significantly higher rate than the Tsp-1-/- mice.

Another characteristic of Itgb6-/-mice is their eventual development of emphysema (Morris et al., 2003). In the alveolar macrophages of Itgb6-/- mice, MMP12, an enzyme strongly associated with the pathogenesis of emphysema, is expressed 200-fold higher than in wild-type counterparts (Houghton, 2015). Although at birth the size of the alveoli is normal, by six months of age the alveolae of Itgb6-/- mice are significantly larger and exhibit mild emphysema, which increases in severity with age (Houghton, 2015).

Mice deficient in Itgb6 develop chronic periodontal disease (periodontitis) (Ghannad et al., 2008), an advanced stage of gingivitis in which the gums pull away from the teeth, leaving space or ‘pockets’ between the teeth and gums that become infected. The consequent immune response leads to degradation of the connective tissue between the teeth and gums. An important interface in the development of periodontal disease is the junctional epithelium (JE), which expresses αvβ6 and is involved in adhesion of the gingiva to the teeth. By 12 months of age, Itgb6-/- mice develop abnormal JE, exhibit periodontal pockets and show evidence of inflammation and bone degradation (Ghannad et al., 2006; Bi et al., 2017).

Itgb6-/- mice also develop amelogenesis imperfecta, a disorder affecting the development of teeth (Mohazab et al., 2013). Amelogenesis imperfecta causes a range of symptoms, including abnormally small teeth prone to breakage and discolouration. Cases of amelogenesis imperfecta have also been observed in clinical studies in patients with ITGB6 mutations (Wang et al., 2014; Poulter et al., 2014).

Clinical Cases of ITGB6 mutations

The first clinical case associated with an ITGB6 mutation was documented in 2013, in which a 7-year-old girl presented with enamel malformations but was otherwise asymptomatic (Wang et al., 2014). Following whole genome sequencing, it was revealed that she had inherited 2 distinct missense mutations in ITGB6. As part of the same study, a second child displaying symptoms of amelogenesis imperfecta was examined and he too had a bi-allelic ITGB6 mutation. Poly-morphism Phenotyping (Polyphen) analysis indicated that both mutations were of high probability of affecting β6 protein structure and causing disease. These and other cases of ITGB6 mutations have been documented across a number of conditions (Table 1).

These cases were the first reported examples of mutations in the ITGB6 gene causing amelogenesis imperfecta. Interestingly, they reported no other clinical symptoms, such as pulmonary inflammation as reported in mouse models. However, it is unknown whether the authors performed a detailed clinical examination of the children. Similarly, further reported cases of amelogenesis imperfecta with ITGB6 mutations failed to disclose other possible clinical symptoms, or whether these were investigated (Poulter et al., 2014).

There is a collection of clinical cases where a large deletion in the 2q24 region encompassing the ITGB6 gene has resulted in respiratory complications in the patient (Takatsuki et al., 2010). These authors described a case of a 2-month-old girl who, among other symptoms,
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Table 1

| Patient | Mutation | Location | Protein Change | Clinical Presentation | Publication |
|---------|----------|----------|----------------|-----------------------|-------------|
| 7 year old female | c.427 G > A | Exon 4 | A143T | Amelogenesis Imperfecta | Wang et al., 2014 |
| 8 year old male | c.1846C > T | Exon 11 | R616X | Amelogenesis Imperfecta | Wang et al., 2014 |
| 3 sisters; aged 7,9 and unknown | c.586C > A | Exon 4 | P196T | Amelogenesis Imperfecta | Poulter et al., 2014 |
| 20 year old female | Deletion | 2q23q31 | n/a | Cognitive and dysmorphic features. Deceased. | Shabtai et al., 1982 |
| 2 year old male | Deletion | 2q23q24.3 | n/a | Dysmorphic features, congenital heart defects. Deceased. | Maas et al. 2000a,b |
| 3 year old female | Deletion | 2q24.1q31.1 | n/a | Dysmorphic features, developmental delay and seizures. Deceased. | Langer et al. 2006 |
| 9 year old male | Deletion | 2q23q31 | n/a | Cognitive and seizures | Grosso et al. 2008 |
| 2 month old female | Deletion | 2q24.2q24.3 | n/a | Severe pulmonary emphysema, seizures, delayed growth and dysmorphic features | Takatsuki et al. 2010 |
| 3 siblings; Male aged 40; Females aged 39 and 33 | n/a | n/a | n/a | Amelogenesis Imperfecta | Ansar et al. 2015 |

Developed severe pulmonary emphysema. After confirming ITGB6 was deleted, they hypothesised that there was a relationship between this deletion and the respiratory complications experienced by the child. However, it is estimated that a total of 34 genes were affected by this deletion. Thus, her emphysema may not have been caused by the ITGB6 deletion alone, despite the similarities in phenotype with the I6gb6-/- mice. A further three cases (Shabtai et al., 1982; Moller et al., 1984 and Langer et al., 2006) document patients with large deletions in the 2q24 region that included ITGB6, two of which resulted in fatalities caused by respiratory complications.

A novel homozygous missense mutation in ITGB6 was found in three members of a Pakistani family presenting with alopecia, intellectual disabilities, and symptoms consistent with amelogenesis imperfecta (Ansar et al., 2016). It is perhaps worth noting that cognitive disabilities have not been noted in experimental mouse studies of I6gb6. The mutation was found in a highly conserved region of ITGB6 which was predicted by in silico analysis to cause significant change to β6, which may its function. Thus, humans with loss of function ITGB6 mutations exhibit some of the phenotypes observed in I6gb6-/- mice (alopecia, amelogenesis imperfecta, periodontitis, and emphysema) but this seems to vary between individuals, presumably due to the clinical severity of the different mutations. Further clinical exploration will result in a clearer picture of how specific mutations manifest.

Conclusions

Although a substantial amount of work has been performed to determine the structure of the ITGB6 gene, there are many unanswered questions as to how β6 protein expression is regulated. Of note, limited work has been performed to further our understanding of post-transcriptional regulation of ITGB6, which likely plays a significant role in αvβ6 expression and biology. Furthermore, investigations of clinical cases involving ITGB6 mutations have not been comprehensive and it is possible we have missed some understanding of the human phenotype that would help us fully appreciate the role of ITGB6 in humans.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Author Contributions Statement

Both authors contributed equally to the conception and writing of the manuscript.

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