

DEF6 Expression and Regulation in Cancer, Chronic Inflammatory Diseases and Autoimmune Diseases: A Review

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Abstract

Def6 protein has been highly associated with autoimmune diseases and become one of risk gene in systemic lupus erythematosus (SLE) disease. Def6 deficiency is spontaneously affecting the development of systemic autoimmune disorder in mice as well as in human. Def6 or IRF-4-binding proteins (IBP) that mostly found among lymph node and thymus are associated with cell survival and cell proliferation. Def6 protein as a novel biomarker has many important functions and roles in human immune system. Def6 is believed to be a potential therapeutic pharmacological target or/and potential drug for some malignancy diseases and pathological disorders. Current studies reveal Def6 expression and regulations not only are found in autoimmune diseases but also in human cancer and chronic inflammatory diseases. High expression of Def6 is correlated with invasive tumours and malignant stage of various cancers.

This review article sums up Def6 expression and regulation in some diseases stated above. Collectively, this review article provides novel studies concerning Def6 as a protein, its correlation with other genes, its mechanism and its expression in some diseases, particularly in cancer, autoimmune diseases and chronic inflammatory diseases.

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Guanine nucleotide exchange factor (Def6) protein introduction

Def6, also known as IRF-4-binding proteins (IBP) or SWAP 70-like adaptor of T cells (SLAT). After first studied by Marc Hotfilder et al. in 1999, Def6 was believed to contribute towards DNA recombination for T and B cell function and lymphoid development and/or function. They also observed that Def6 in human was predominantly expressed in haematopoietic tissues, lymph node, thymus and peripheral blood leucocytes, but in mouse it was highly expressed in spleen. Def6 expression also performed to be down-regulated upon erythrocyte, macrophage and granulocyte differentiation¹. Def6 is down-regulated upon myeloid differentiation together with erythroid heredities and found to be closely related but not

equal with protein involved in B cell antigen receptor complex-switch associated protein 70 (SWAP-70)^{1,2}.

Further study in 2003 placed Def6 as a guanine nucleotide exchange factor for Rho-family GTPases signalling pathways, an intracellular protein located in human lymphoid tissue, chromosome number 6, p arm 21 number 31 (6p21,31). Def6 inversely expressed in factor-dependent cell progenitors (FDCP 6 homolog) that is highly expressed in B and T cells and play critical role in the immune system³⁻⁶. Def6 is also predicted to be promptly associated with cell survival. Def6 deficiency displayed disability of T cells in performing apoptosis through a cell autonomous pathway⁷.

Def6 protein as a converter expressed in hematopoietic system interacts with and alters the function of small GTPase Rac1 in fibroblasts. Def6 or SLAT is highly expressed in macrophages prevents the IgG Fcγ receptor-mediated phagocytic ability of T helper 1 cells. In bone marrow-derived macrophages, Def6 protein is engaged to the initial phagosomes formed via

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Fcγ receptor arrangement. Def6 or SLAT turns as a gatekeeper for the amount of Rac recruited to the phagosomes formed by Fcγ receptor arrangement and thus is capable of regulating F-actin restructuring and subsequently phagocytosis⁸. In human kidney, Def6 protein is highly expressed in glomeruli, followed by tubules and acts as a novel regulator of small GTPases in podocytes⁹.

Def6 unveils substantial homology to only one other protein, SWAP-70. SWAP-70 and Def6 constitute the SWEF family, a unique family of Rho GTPase-regulatory proteins that control both cytoskeletal dynamics and interferon regulatory factor-4 (IRF4) activity. Recent study conducted by Manni et al. (2018) revealed that several polymorphisms in Def6, which is located centromeric to the locus encoding the major histocompatibility complex²⁶, together with population expansion of age-associated B cells. ABC-like cells and aberrancies in interleukin-21(IL21) –IL21R or IRF5 have also been detected in other systemic autoimmune conditions such as rheumatoid arthritis and inflammatory bowel disease¹⁰. SWAP-70 and Def6 inhibit the cytokine IL-21-induced transcription factor IRF5 from binding to DNA and engaging the transcription factor T-bet and thus diminish the activation of ABC like cells¹¹.

Def6 expression in autoimmune diseases

Elevated Def6 expressions were commonly observed in autoimmune diseases such as experimental autoimmune encephalomyelitis (EAE), experimental autoimmune uveitis (EAU), systemic lupus erythematosus (SLE) and psoriasis vulgaris^{7,12-16}. Def6 is involved in autoimmunity through regulation of encoding interferon regulatory factor 4 (IRF4) in IL-12 responsiveness¹⁷. Study from Sun et al., in 2016 found Def6 as one of new loci which is associated with SLE in individuals with an Asian ancestry and Def6 as a potential drug target for SLE therapy¹⁸.

Associations between SLE and variants of IL-21, IL-21R, DEF6 and IRF-5 have all been acknowledged in genome-wide association studies. These indicate the intriguing possibility that inappropriate regulation of this pathway plays crucial role in the pathogenesis of SLE¹⁰.

Def6 expression in various cancers

Recently, Def6 overexpression are also observed in many malignancies such as human renal cell carcinoma, extraskelatal myxoid chondrosarcoma, human colorectal cancer, human breast cancer, ovarian carcinoma and in oral squamous cell carcinoma as well^{4,5,19-24}. Besides activating Rho-family GTPases, Def6 also collaborates with activated Rac1 to exert its cellular role and ends up in regulating cell morphology²⁵. Def6 controls the migration, invasion, matrix metalloprotease production in breast cancer cells, actin cytoskeleton reorganization and the initiation of GTP-Rac1, GTP-RhoA and GTP-Cdc42⁵. Def6 definitely has major role in pathogenesis of cancers through many pathways.

Def6 is highly preserved in vertebrates and acts as a guanine nucleotide exchange factor for Rho-family GTPases, including Rac1, Cdc42 and RhoA^{5,26} that involved cytoskeletal organization, cell cycle progression and extracellular signal transduction, as equal to that of in the cancer cells proliferation, migration, invasion and metastasis of cancer cells²⁷⁻³⁰. Otsubo et al. (2014) found that Def6 is highly expressed in human renal cell carcinoma and contributes to enhance tumour angiogenesis in renal cancer patients³¹. Def6 might have a crucial regulation in tumour invasion and metastasis and might serve as targets for antiangiogenic therapy of cancer patients. Moreover, another Def6 studies also reveal another function of Def6 in other systems besides immune cells such as in muscle cells and stimulates differentiation of myoblast cells³².

Def6 expression in chronic inflammatory diseases

Not only involved in some cancers and autoimmune diseases, increased expression of Def6 was also detected in some inflammatory diseases and affecting pathological bone resorption³³. Osteoclasts that produced by monocyte/macrophage precursors are the exclusive cell type responsible for bone resorption. Osteoclastogenesis is effectively induced by the major osteoclastogenic cytokine receptor activator of NF-κB ligand (RANKL). In chronic inflammatory diseases such as rheumatoid arthritis (RA), psoriatic arthritis, periprosthetic loosening and chronic periodontitis, osteoclastogenesis and osteoclast-

mediated bone resorption are increased, causing bone destruction as a main cause of morbidity and disability³⁴⁻³⁸. Def6 functions as an inhibitor of excessive osteoclast formation and bone destruction in RA. Def6 pathway signifies a novel therapeutic target to avoid pathological bone destruction in chronic inflammatory diseases³⁹. These studies evidenced Def6 could be target therapy in chronic inflammatory diseases through inhibiting osteoclastogenesis.

Def6 regulations in autoimmune diseases

A study about Def6 in experimental autoimmune encephalomyelitis (EAE) conducted by Joshi et al. in 2017 revealed the role of Def6 in development and pathogenesis of EAE by some aspects. Def6 deficiency leads to EAE resistance and hypo responsiveness of CD4+ cells. Absence of Def6 leads to impaired myelin oligodendrocyte glycoprotein (MOG)-specific T helper 1 and 17 cells response. Moreover, Def6 is intrinsically required for T helper 17 cell differentiation mechanism⁴⁰.

Recent study regarding Def6 role in systemic lupus erythematosus (SLE) in mice was conducted by Yi et al., in 2017. They stated that Def6 deficiency in SLE leads to the progress of SLE since it related to Def6 contribution in controlling protein synthesis. As a consequence, it will lead to T cells dysfunction and promote autoimmune. Def6 is highly expressed by naive T helper cells. Absence of Def6 protein and SWAP70 implies to the development of SLE, which occurred predominantly in female mice, as well as in human SLE. Mice lacking of Def6 protein produce deregulation of Bc16 protein synthesis in T cells. Furthermore, lacking of Def6 will impulsively develop a systemic autoimmune disorder that characterized by elevated expression of effector T cells, germinal centre (GC) B cells proliferation and production of autoantibody^{17,41}.

Def6 regulations in malignancy diseases

Study conducted by Zhang et al. in 2014 about Def6 in breast cancer found that Def6 has some contributions in carcinogenesis through some mechanisms. First, Def6 promotes the phenotype of cancer cells through epithelial-to-mesenchymal transition (EMT) alteration and cellular motility. Secondly, Def6 is upregulated in

epidermal growth factor (EGF)-induced EMT. Third, Def6 silencing inactivates epidermal growth factor receptor (EGFR) signalling in carcinoma cell lines. Forth, Def6 silencing reduces the expression of EMT-induced transcription factors. Fifth, Def6 promotes cancer cell migration; invasion of MDA-MB-231 and HS578 cells and matrix metalloprotease (MMP) production. Lastly, Def6 promotes actin cytoskeleton rearrangements via Rho-family GTPases signalling pathways including Rac1, RhoA and Cdc42⁵.

In 2012, Jian et al. revealed that Def6 was expressed ectopically in some of oral squamous cell carcinoma (OSCC) tissues but not in normal oral mucosa tissues. Def6 plays an important role in the carcinogenesis of OSCC through these mechanisms, such as by shortening the G1 interval in the cell cycle, stimulating Tca8113 cell proliferation and increasing the expression of cyclin D1, an important regulator of the G1-S alteration⁴.

Another study in Def6 by Yang et al. in 2012 identified Def6 as a direct transcriptional target of p53 tumour suppressor gene through some mechanisms, which are suppressing the DNA damaging agents that express wild type p53, decreasing cisplatin-induced breast cancer cell apoptosis in MCF-7 cells and increasing cisplatin resistance through loss of p53 function and overexpression of antiapoptotic Bcl-2. Other than that, Def6 also negatively regulates p53 expression. p53 is highly related with various cancer including oral cancer⁴². Tumour suppressor gene p53 is directly targeted p53R2 as one of ribonucleotide reductase (RNR) subunit in assisting DNA synthesis and DNA repair mechanism. Thus, could be an effective target for cancer therapy⁴³. Any alteration regarding p53 expression may lead to DNA damage and abnormal cell proliferation which will contribute to cancer development⁴⁴.

Def6 regulations in chronic inflammatory diseases

Besides in cancer and autoimmune disease, Def6 also regulates bone resorption in some chronic inflammatory diseases through these mechanisms^{39,45,46} includes regulates the macrophage responsiveness to receptor activator of NF- α B ligand (RANKL), inhibits tumour necrotizing factor (TNF)- α -induced osteoclast formation, TNF- α regulates Def6 expression in

human monocytes/ macrophages. In addition, Def6 suppresses the c-Fos-Blimp/NFATc1 axis expression by regulating an endogenous IFN- β -mediated-autocrine-inhibitory loops.

In line with Joshi et al. (2017), other studies conducted by other researchers reveal some mechanisms of Def6 in immune system particularly with T and B cells. There is a pivotal role of Def6-mediated NFAT activation in CD8 + T cells and providing new insight into the precipitation pathway elaborated in CD8 + T cell proliferation. Furthermore, Def6 are required in thymic DN1 cell expansion, T cell activation, and Th1 and Th2 inflammatory responses. Additional critical function of Def6 is for TCR-induced adhesion to ICAM-1 and affinity maturation of LFA-1 in Cd4 (+) T cells⁴⁷⁻⁴⁹.

Table 1 above shows Def6 studies in various methods used in detecting Def6 expression and its research findings. Immunohistochemistry method is mostly used in detecting Def6 protein^{4,5,12,15,21,31,50,51}. This might happen due to its specificity, sensitivity and effectiveness. Second method mostly used in detecting Def6 is PCR, since PCR has high sensitivity and specificity⁵².

All researchers are managed to detect Def6 expression in their samples and agreed that Def6 plays role in autoimmune diseases, cancer and chronic inflammatory diseases. In additional, Def6 found to be as a potential therapeutic agent and selective target for therapy of cancer, autoimmune diseases and chronic inflammatory

diseases^{4,14,31,40,54}. Def6 that mostly found in haematopoietic system acts as a novel activator in Rho GTPases which are crucial in innate immune system^{9,55}.

Conclusions

Studies show the Def6 expression and role of Def6 protein in autoimmune diseases, chronic inflammatory diseases and numerous cancer diseases. Def6 is potential as a therapeutic pharmacological target or therapy agent of some diseases particularly as an antiangiogenic and cancer therapy. Further studies in future may reveal Def6 protein as a potential biomarker for prognostic tool and precise treatment for autoimmune diseases, chronic inflammatory diseases and cancer.

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Declaration of Interest

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Researcher	Ref	Sample	Methods	Findings
Purwaningsih et al., 2019 (Malaysia)	23	Human oral cancer	Immunohistochemistry	More than 80% of Def6 were found in oral cancer, but none in normal oral mucosa.
Joshi et al., 2017 (Sweden)	39	Human peripheral blood cells	qRT PCR, Phosphoproteomics, coimmunoprecipitation, western blot	Def6 is a selective target for T cell-mediated inflammatory and autoimmune diseases.
Yi et al., 2017 (USA)	40	Mice	Western blotting and immunoprecipitation, DNA constructs	Absence of Def6 protein and p26 can result in T-cell dysfunction that leads to autoimmune diseases, such as SLE.

Liew et al., 2016 (Taiwan)	18	Human ovarian carcinoma	Western blot assay	Def6 detection may be used as an independent prognostic factor. Def6 expression was associated with poor prognosis in human ovarian carcinoma.
Otsubo et al., 2014 (Japan)	19	Human renal cell carcinoma	Microarray gene expression analysis, PCR and immunohistochemistry	Def6 is upregulated in hTEC and in vivo in tumour vessels of renal cell carcinoma. Def6 might contribute towards tumour angiogenesis enhancement and might serve as targets for antiangiogenic therapy for cancer patients.
Zhang et al., 2014 (China)	21	Human breast cancer	Immunohistochemistry, immunofluorescence, western blot, invasion and migration assay, GTPase activity assay, and RT PCR	Def6 controls the migration, invasion, matrix metalloprotease production in breast cancer cells, actin cytoskeleton reorganization and the initiation of GTP-Rac1, GTP-RhoA and GTP-Cdc42.
Yang et al., 2012 (China)	53	Human breast cancer	Western blot, RT-PCR, electrophoretic mobility shift assays (EMSA), chromatin immunoprecipitation assay (ChIP), RNA interference	Relationship between Def6 signalling and p53 tumour suppressor gene shows that Def6 is potential as a target for pharmacologic intervention of human breast cancer resistant to cisplatin therapy.
Jian et al., 2012 (China)	4	Oral squamous cell carcinoma (OSCC)	Flow cytometry, western blot, MTT assay, colony formation assay, immunohistochemistry, immunofluorescence assay, cell culture, tumour xenografts	Def6 or IBP expression is significantly correlated with tumour size, differentiation, clinical stage and distant metastasis of OSCC. Def6 might be as potential therapeutic agent for OSCC.
Vistica et al., 2012 (USA)	13	Experimental autoimmune uveitis (EAU)	ELISA and PCR	Def6 plays a major role in the development of EAU and related immune response. Def6 could be a potential drug target for T cell-mediated autoimmunity and inflammation.

Ni et al., 2012 (China)	14	Psoriasis vulgaris	Immunohistochemistry	Def6 might be involved in the development of psoriasis vulgaris.
Zhang et al., 2009 (China)	5	Human colorectal cancer	Western blot and blocking experiment, immunohistochemistry, and RNA and RT PCR	Def6 expression is correlated with clinical stage of patient and level of differentiation of cancer cells. Def6 is postulated as tumour marker and therapeutic target for colorectal cancer.
Li at al., 2009 (China)	20	Breast cancer	Cell culture, immunohistochemistry, tissue microarrays (TMA)	Def6 expression is correlated with degree of malignant breast tumour. Def6 contributes in tumourgenesis of breast cancer. Def6 is a potential biomarker for invasive breast tumour.
Canonigo-Balancio et al., 2009 (USA)	11	Mice	Immunohistochemistry	Def6 plays role in controlling autoimmune and inflammatory diseases by regulating the response of T helper 17 cells. Def6 is involved in initiation and progression of experimental autoimmune encephalomyelitis (EAE).
Subramanian et al., 2005 (USA)	15	Extraskelital myxoid chondrosarcoma (EMC)	Tissue microarrays	Def6 was one of the top five genes expressed in EMC. Def6 has a role in pathogenesis of EMC.
Hotfilder et al., 1999 (UK)	1	Mouse genes Human genes	Northern blot analysis, PCR, DNA sequencing	Def6 mostly found in lymph node, thymus and peripheral leucocytes. Def6 expression closely correlated with lymphoid development and/or function

Table 1. Def6 studies in different methods.

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