

## REVIEW ARTICLE

### Epidermal Growth Factor-Like Domain-8 (EGFL8) in Mammals: The Story so far

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#### ABSTRACT

Epidermal growth factor-like domain-8 (EGFL8) also known as vascular endothelial statin-2 (VE-statin-2). It was identified by using retroviral gene entrapment vectors, expressed in endothelial cells. It is located on chromosome 6 in humans and chromosome 17 in the mouse. EGFL8 codes a protein of 293 amino acids with an amino-terminal signal peptide and has two EGF-like domains. EGFL8 plays a very important regulatory role in thymopoiesis, cell migration and invasion through the modulation of Notch signaling. Although the signaling regulatory factors of EGFL8 need to be explored but recent scientific advances have revealed some important aspect of its regulation. This review summarizes the current knowledge about all aspects of EGFL8 since its discovery.

**Key Words:** *Endothelial, EGFL, EGFL8, Notch, Thymopoiesis.*

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#### Introduction

Epidermal Growth Factor-like (EGFL) proteins contain various repeats. Their receptor binding modulates a variety of life functions, including proliferation, differentiation, apoptosis, adhesion and migration.<sup>1,2</sup> The expression of EGF receptors has been found in many types of cells such as osteoblasts, osteoclasts, and endothelial cells. There are two types of EGF-like family members that are membrane-bound (EGFL2, EGFL5 and EGFL9) or secreted proteins (EGFL3, EGFL6, EGFL7 and EGFL8).<sup>3,4</sup> These proteins are involved in the regulation of angiogenesis.<sup>5</sup>

Notch genes, code for transmembrane receptors, which regulate cell fate and control cell division in both mammals and lower organisms. In mammals, there are four different Notch receptors from Notch-1 to Notch-4. The Notch receptors contain a single transmembrane protein, which is composed of 29 to

36- EGFL repeats within the extracellular domain.<sup>4</sup> The interactions of Notch and EGFL family members have been identified in recent studies. One of the studies showed the binding of EGFL7 to Notch family receptors acts as an antagonist of the Notch signaling pathway in cultured neural stem cells.<sup>6</sup> One of the recently identified members of EGFL family includes EGFL8, which shares sequence as well as functional similarity to the EGFL proteins EGFL8 is reported to regulate T cells, thymic epithelial cells, embryonic development and show a modulated expression in cancerous tissues.

#### 1. Discovery, expression and tissue distribution of EGFL8

EGFL8 was first recognized in 2004 and was characterized for its high resemblance with EGFL7. It was identified in the mouse genome for the first time with a Basic Local Alignment Search Tool (BLAST) search technique using the EGFL7 amino acid sequence. It showed a paralog of EGFL7 encoding a protein of 293 amino acids which is positioned on mouse chromosome 17 (GenBank accession no. NM 152922). EGFL8 shares about 35% identity with EGFL7 and both proteins consist of Ca<sup>2+</sup> binding, a N-terminal signal peptide and two EGF-like domains.<sup>7</sup> The EGFL family members are classified into two groups. Bioinformatics analyses showed transmembrane domains within the EGFL2, EGFL5 and EGFL9, whereas EGFL3, EGFL6, EGFL7 and EGFL8

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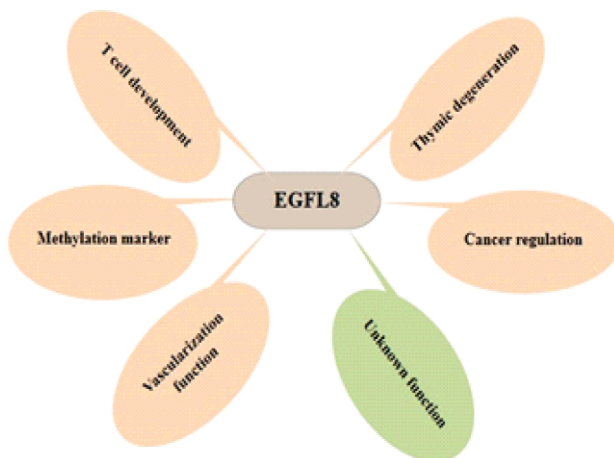
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are without transmembrane domains.<sup>8</sup> The expression level of EGFL8 was identified in a number of adult mouse tissues including skin, adrenal glands, urinary bladder, ductus, lymph nodes, testis, ovary, penis, epididymis, spleen, liver, intestine, kidney, brain, thymus, lung, muscle, ovary, heart and also an increasing expression level during embryonic development.<sup>7</sup> Another study showed expression of EGFL8 both in osteoclastic and osteoblastic-like cells by using microarray and semi quantitative RT-PCR analysis. It is identified that high expression of EGFL8 in both osteoblastic and osteoclastic like cells is comparable with other EGF-like family members termed as EGFL2, EGFL3, EGFL5, EGFL6, EGFL7, and EGFL9.<sup>8</sup> This wide range expression of EGFL8 suggesting that it plays a vital role during physiologic and pathologic regulation.

For a more detailed study EGFL8 was overexpressed and knockdown in thymic epithelial cell line with siRNA (small interfering RNA).<sup>9,10</sup> The overexpression and knockdown of EGFL8 in TEC play a more regulatory function in thymic epithelial cells and T cell development in the thymus.

## 2. Functional Background of EGFL8

The multiple known targets of EGFL8 are shown in Figure 1. EGFL8 expression has been observed in gastric cancer and is correlated with elevated tumor, node, metastasis stage, and poor prognosis in gastric as well as in colorectal cancer.<sup>11,12,13</sup> The thymus is the fundamental lymphoid organ that provides a suitable microenvironment for T-cell development and maturation.<sup>14</sup> The required differentiation and



**Fig 1: Diverse functional target of EGFL8. The EGFL8 are known to influence the T cell development, thymic epithelial regulation, Cancer regulation, Vascularization and identified as methylation marker**

organization of different TECs (thymic epithelial cells) is critical for both thymocytes expansion and T-cell repertoire selection.<sup>15</sup> The negative regulatory effect of EGFL8 on mouse thymic epithelial cells and thymocytes, and T-cell regulation has been shown, by construction of both EGFL8 overexpressed stable cell line as well as production of mouse recombinant protein. EGFL8 downregulates the thymic epithelial expression of ICAM-1 as well as IL-7, GM-CSF and TECK. The inhibitory effect of EGFL8 was recovered with knockdown of EGFL8 with EGFL8 targeted siRNA in epithelial cells of mouse thymus. EGFL8 which is a paralog of EGFL7 has an inhibitory effect on downstream target of Notch receptors, Hes1 and Hey1.<sup>9,10</sup> It is well known that Notch signaling mediates cell fate determination through the regulatory, inhibitory and inductive action. Notch signaling play a key role in embryogenesis of many organ systems, which was verified in Notch1, Notch2, Jagged2, and Delta1 knockout mice.<sup>16</sup> Other than normal cells or developmental regulation, Notch signaling regulates the balance among cell proliferation, differentiation, and apoptosis. It may be linked with pancreatic cancer,<sup>17</sup> melanoma cells<sup>18</sup> and ovarian cancer.<sup>19</sup> The  $\gamma$ -Secretase inhibits Notch by cleaving the Notch intracellular domain (NICD) that prevents the generation of the intracellular domain of Notch molecules. However, the current challenge of the  $\gamma$ -secretase inhibitors are its cytotoxic side effects, which need to be addressed by studies that can help design targeted therapies against specific mutations in the *Notch* gene while reducing adverse side effects.<sup>20</sup> Recent data has shown that secretory EGFL7 is a functional antagonist of Notch receptors and interacts with all four subtypes of Notch receptors.<sup>6</sup> The effect of EGFL8 on  $\gamma$ -Secretase activation is still to be determined in the context of Notch downstream signaling inhibition.

Another study described EGFL8 as a consistent methylation marker in different human tissues samples. A bioinformatics approach generated 27 genes showing consistent methylation levels across all samples. Among them, the peak five genes (N4BP2, EGFL8, CTRB1, TSPAN3, and ZNF690) were analyzed using mass spectrometry in 24 human cell lines. Thus, EGFL8 was highly methylated both in tissues samples and 24 cell lines across 13 tissue types examined by mass spectrometry.<sup>21</sup> Epigenetic

processes such as hypermethylation play a regulatory role in the cell differentiation, chromatin structure; however the abnormal methylation in genes can affect the mechanisms of various vital biological functions. It is hypothesized that hypermethylation might regulate tumor development and progression.<sup>22</sup> EGFL8 can be a potential new target gene for regulating tumor development and progression. Further exploratory studies on regulatory functions of EGFL8 methylation status in cancer are required, as currently little information is available on its molecular characterization.<sup>23</sup>

### 3. Future Prospects

The involvement of EGFL8 cannot be ignored in multiple signaling pathways such as Wnt/ $\beta$ -catenin, Ras, NF $\kappa$ B, Notch. The literature review has shown that regulating the function of EGFL8, can play an important role in various important biological functions such as, immunity, inflammation, Graft versus host disease (GvHD), regeneration, wound healing and embryonic development. EGFL8 may be a future therapeutic target for various pathological conditions.

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