胞红蛋白结构、功能及相关机制

周丹蕾^{1,2}, 刘小平^{2*}, 罗爱芹^{1*}

1. 北京理工大学生命学院, 北京 100081;

2. Davis Heart and Lung Research Institute and Division of Cardiovascular Medicine, Department of Internal Medicine, College of Medicine, The Ohio State University, Columbus, OH 43210, USA

* 联系人, E-mail: liu.547@osu.edu; bitluo@bit.edu.cn

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摘要 胞红蛋白(Cygb)是一种血红素蛋白, 属于珠蛋白家族中的一员. Cygb主要在纤维母细胞、心肌细胞及血管平滑肌细胞中表达, 其主要生理功能还不清楚. 据文献报道, 胞红蛋白可储存氧、去除活性氧毒性、参与胶原合成、代谢一氧化氮、抗纤维化、调控基因表达、转导信号、抗肿瘤、调节血压. 本文重点对胞红蛋白的结构特性、主要功能及作用机制展开论述, 并介绍了胞红蛋白与疾病的关系, 以期今后的研究提供理论基础.

关键词 胞红蛋白, 结构, 功能, 作用机制

珠蛋白家族是一类在细菌、真菌、植物和动物中普遍存在的含血红素的珠状蛋白质. 它们具有相似的一级结构和三级结构, 都能在血红素活性中心的亚铁上可逆地结合氧分子, 具有储存和传递氧的作用. 生物体在低氧情况下可以诱导这类蛋白的表达^[1]. 珠蛋白具有传递电子的作用, 它们可以通过血红素中Fe二价与三价间的变化呈现出还原态或氧化态^[2]. 此外, 它们还能在细胞内进行信号传导^[3,4], 是一类在生物体内有重要作用的血红素蛋白.

近年来, 一些新的珠蛋白不断被发现, 除了最老的血红蛋白和肌红蛋白, 其他珠蛋白在脊椎动物中相继被发现, 因此, 珠蛋白家族同源关系的传统观念也在不断修正. 研究者发表了越来越多的珠蛋白基因组序列数据^[5]. 目前在高等脊椎动物中至少已经发现了4种

常见珠蛋白: 血液中的血红蛋白(hemoglobin, Hb)、肌肉组织中的肌红蛋白(myoglobin, Mb)、神经元和神经内分泌组织中的脑红蛋白(neuroglobin, Ngb)^[6], 以及在多器官中发现的胞红蛋白(cytoglobin, Cygb)^[7,8]. 此外, 还有4种珠蛋白随后陆续被发现, 在寄生虫中发现的珠蛋白X (Globin X, GbX)^[9], 非洲爪蟾(Xenopus laevis)中发现珠蛋白Y (Globin Y, GbY)^[10], 鸡眼睛中发现珠蛋白E (Globin E, GbE)又叫eye-globin^[11]. 最近发现的嵌合珠蛋白Androglobin (Adgb), 其N末端有与钙蛋白酶类似的结构区, 内部有珠蛋白结构^[12].

血红蛋白以四聚体的形式广泛存在于生物界中, 在脊椎动物中主要存在于血液的红细胞中, 由两对不同的珠蛋白链(α 链和 β 链)组成, 在血液循环中负责携带和运输氧, 有代谢一氧化氮(nitric oxide, NO)的酶活

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性^[2,13]。肌红蛋白是一种在心脏和肌肉细胞中表达最多的单分子蛋白^[14], 对氧气亲和力大于血红蛋白, 主要是储存氧, 也有促进氧扩散, 运输氧给线粒体和多种氧化还原酶的作用^[15]。脑红蛋白主要在脊椎动物大脑中表达, 其中人和小鼠(*Mus musculus*)的脑红蛋白是以单体形式存在, 和肌红蛋白比起来, 为了增加可用氧, 脑红蛋白对氧有更高的亲和力^[6], 可以促进氧向线粒体扩散, 保护神经细胞免受缺氧缺血的损害。同时脑红蛋白也能清除活性氧(reactive oxygen species, ROS), 有NO加双氧酶(NO dioxygenase, NOD)的活性, 可以将NO氧化为硝基NO₃⁻^[16,17]。胞红蛋白首次被报道于2001年, 是由日本学者Kawada和同事在研究大鼠(*Rattus norvegicus*)肝星状细胞激活的分子机制时发现的。起初, 此蛋白被命名为星状细胞激活蛋白(stellate cell activating protein STAP)^[18]。后来在2002年, 美国科学家Trent和Hargrove^[19]发现, 胞红蛋白是在人体组织内广泛表达的一种6配位蛋白, 并将其指定为组胺球蛋白(histoglobin)。同年, 德国学者Burmester等人^[7]在小鼠、人体和斑马鱼(*Danio rerio*)内也发现了胞红蛋白并通过基因测序的方法证实小鼠和人的胞红蛋白包含190个氨基酸, 斑马鱼有174个氨基酸, 生物进化分析表明其与Mb, Hb, Ngb属于同一家族, 并正式命名为胞红蛋白。

胞红蛋白(cytoglobin, Cygb)广泛分布于心、胃、膀胱及小肠等人体组织中, 从细胞水平来看, Cygb在心肌细胞、成纤维细胞、血管平滑肌细胞中均有表达^[20]。由于其具有储存氧, 参与胶原合成、代谢NO, 清除细胞内ROS及基因调控等多种功能, 尤其是文献报道Cygb有抗纤维化、抗肿瘤、调节血压和血管阻力的作用, 因而引起了研究人员的高度关注。本文试对Cygb的结构特性、功能、作用机制及与疾病的关系等展开综述, 为进一步研究Cygb做铺垫。

1 胞红蛋白结构分析

通过对基因序列和基因结构分析, Cygb位于染色体17q25上, 为单拷贝基因, 包含3个内含子和4个保守的外显子。Cygb由190个氨基酸组成, 主要以单体形式存在于体内, 分子量为21 kD^[7]。与经典的珠蛋白折叠构象类似, Cygb具有三明治夹心型 α -螺旋珠蛋白折叠结构^[21]。Cygb的活性中心Fe²⁺或Fe³⁺有6个配位键, 其

中4个键是与4个吡咯环中的4个氮原子配位结合, 这4个吡咯环通过4个甲炔基相连形成环状, 两个组氨酸残基His113(F8)和His71(E7)分别与Cygb的血红素-铁结合形成His-Fe-His轴向配位^[21]。尽管Cygb具有与其他珠蛋白家族成员类似的基因和蛋白结构, 其基因序列与其他珠蛋白仍有不同。哺乳动物的Cygb在其两末端各延长20个氨基酸^[7,19,22], 其结构及关键末端残基如图1所示。

在Cygb分子结构中, 其核心区有150个氨基酸构成, N-和C-末端各有约20个氨基酸残基的两个延伸片段。Makino等人^[24]研究显示, A螺旋之前的N-末端残基(4~20)中存在额外螺旋并且C末端区域(168~188)存在有序的环境结构。核心区(由血红素辅基与G螺旋和H螺旋所围)内形成一个大的非极性穴, 穴通过球蛋白G与H螺旋之间一个狭小隧道与蛋白的外表面相通。此穴为配体扩散至血红素或离开血红素提供了途径, 也是配体暂时结合的部位^[25]。与Hb和Mb不同, 脱氧状态Hb和Mb的血红素铁离子呈五配位结构, 而Cygb类似于Ngb, 在第六配位无外源性配体时, Fe离子的第六配位位置由远位His残基(E7)占据, 形成His-Fe-His的六配位体^[26,27]。

氧气(O₂)、一氧化碳(carbon monoxide, CO)或NO等外源配体能够取代远位His残基与Cygb的二价Fe离子(Fe²⁺)配位, 但这些外源小分子配体在与Cygb结合之前必须先与内源性配体HisE7进行竞争。Cygb蛋白在C38和C83处的半胱氨酸能形成分子内二硫键从而影响到远位His残基与Fe²⁺的亲和力。Cygb还显示出非常高的热稳定性, 其DNA的解链温度(T_m)为95°C, 高温下仍具有较高的生物活性^[28]。Cygb在体内主要以单体形式存在, 在体外由于分子间的二硫键存在也可形成二聚体和四聚体^[29-31]。Cygb四聚体在结合氧时与血红蛋白Hb类似, 也存在协同作用^[26]。Tsujino等人^[30]还发现, Cygb聚合体可以改变与外源配体的亲和力, 单体部分对外源配体的亲和力比四聚体部分高几乎1000倍。

Cygb五配位和六配位的转变可以引起构象转变^[23], 这种构象转变与Cygb血红素的环境和外源配体有重要关系^[32]。Makino等人^[33]在连二亚硫酸钠(Na₂S₂O₄)存在的条件下, 在CO氛围中测量人体Cygb的晶体结构发现, CO配体占据了亚铁血红素的第六配位时, His81(E7)的咪唑基团从第六配位断开。Zhang等人^[4]发现, Cygb与外源配体CO结合时, 其环区和内腔受到显

图1 Cygb “three-over-three” α -螺旋体折叠的立体图(8个蓝色条带)(网络版彩图)

关键的末端残基有: 亮氨酸(Leu) B10, 苯丙氨酸(Phe) CD1, 组氨酸(His) E7, 缬氨酸(Val) E11(A)和邻近位点的缬氨酸(Val) F4, 组氨酸(His) F8, 组氨酸(His) FG3(B)^[23]

著影响。AB, GH和EF环都经历显著的重排, 蛋白构象发生变化。接着, Gabba等人^[34]和Pietra^[35]分别研究了Cygb结合CO配体时的动力学模型和内腔结构的变化, 这为进一步研究Cygb的功能做了铺垫。此外, Cygb中Cys38和Cys83二硫键的形成能够调节其构象动力学, 减弱对内源配体的结合, 使Cygb对外源配体有较高的亲和力^[30,36,37]。Astudillo等人^[38]根据CO光解离的热力学曲线提出解离配体的逃逸路线, 在没有二硫键的Cygb中, 配体通过疏水通道快速逃逸; 在形成二硫键的Cygb中, 配体CO通过所谓的“组氨酸门”远端His64逃逸。

此外, 脂类分子也可以和氧化态的Cygb (Fe^{3+})结合, 使Cygb从六配位转到五配位^[39]。该过程只发生在氧化态的Cygb (Fe^{3+})上, 表明氧化态的Cygb与脂类分子结合及其随后的配位转化可能与Cygb的生理功能有关^[40]。

2 胞红蛋白的功能特性及相关作用

目前报道的Cygb具有以下几种功能: 具有过氧化物酶活性^[18], 可以通过清除体内活性氧来减少氧化应激的损伤^[41-44], 保护细胞^[45]并发挥抗癌作用^[46]; 产生或代谢NO, 调节血压^[47,48]; 抑制胶原合成, 抗纤维化^[49]; 可作为潜在的基因药物^[50]; 激活肌源祖细胞, 促进肌肉再生^[51]; 具有结合储存氧功能^[30,37,47,52]。

氧化应激可以增加胶原蛋白合成^[53], 加快肾纤维化过程^[54]。He等人^[55]证明Cygb在体内体外都有抗肝纤

维化的作用。Cygb过表达可以使以前引发的纤维化被修复, 在控制组织纤维化上有很大作用。同时, Cygb在组织损伤时表达上调可以抑制自由基诱导的星状细胞活化和随后的组织纤维化^[56]。Mimura等人^[49]将Cygb亚铁血素部位的His81突变后, 使其在肾脏成纤维细胞中过表达。他们考察了肾脏成纤维细胞的胶原蛋白合成, 发现His81突变后的Cygb减弱了对胶原合成的抑制效果, 说明Cygb中的血红素可以通过氧化应激起到抗纤维化的作用。Beckerson等人^[57]将Cygb末端组氨酸His81突变成H81Y和H81V后, 发现突变体的过氧化物酶活性很低, 减弱了对组织纤维化的抑制。

Cygb被报道可以作为潜在的肿瘤抑制因子, 有抑制肿瘤的活性, 其启动子基因甲基化和肿瘤细胞缺氧可以共同调节Cygb的表达^[58]。Cygb基因分布于染色体17q25上, 在恶性肿瘤中经常丢失, 在肺癌中Cygb基因甲基化会增加^[59]。Shivapurkar等人^[46]发现, 在Cygb阳性表达的肺癌细胞群中, 用RNA干扰技术抑制Cygb基因的表达会导致癌细胞群落增加; 相反, 在Cygb不表达的肺癌和乳腺癌细胞中增加Cygb的表达可以减少癌细胞群落。Fang等人^[60]在研究人神经胶质瘤细胞时发现用抗霉素A作为线粒体电子传递链的抑制剂时, Cygb缺陷的细胞 H_2O_2 含量较高, Cygb过表达的细胞中 H_2O_2 含量显著降低, 说明Cygb可能通过降低氧化应激的水平起到抗肿瘤的作用。Thuy le等人^[61]发现, Cygb基因敲除的小鼠更易被N,N-二乙基亚硝胺(diethylnitrosamine, DEN)诱导肿瘤发生。Xu等人^[62]和Mogal等人^[63]观察到Cygb的低表达与肿瘤复发有显著的相关

性. McRonald等人^[64]报道食管癌活检中的*Cygb*基因表达和正常食管中的*Cygb*基因表达相比减少70%. 说明*Cygb*的表达多少与肿瘤密切相关. Thuy等人^[65]报道在非酒精性脂肪性肝炎和肝癌细胞中*Cygb*表达降低, 在肝组织上, *Cygb*缺陷的小鼠有显著的炎症和纤维化.

*Cygb*除了具有以上功能外, 近些年还发现*Cygb*在体内与NO代谢有关. 首先, Hundahl等人^[66]在表征小鼠脑中*Cygb*的定位时发现在大多数神经细胞中, 神经元NO合酶(nNOS)和*Cygb*共定位, 并在大鼠海马中观察到*Cygb*和nNOS共表达^[67]. 他们的实验结果表明, *Cygb*参与NO代谢, 同时证明了慢性束缚应激可以诱导*Cygb*和nNOS高度的共表达. Gardner等人^[68]研究证明, *Cygb*在体外或肝细胞中都具有NO双加氧酶(nitric oxide dioxygenase, NOD)功能.

Halligan等人^[69]报道了*Cygb*在血管平滑肌细胞和外膜细胞中有表达, 并且可以介导NO代谢. Liu等人^[70,71]也报道了NO在血管中代谢的速度与氧浓度紧密相关, 并证明了*Cygb*介导的NO代谢具有氧依赖性. 后来, Liu等人^[72]对*Cygb*介导的NO代谢动力学进行了深入研究, 并提出了一个详细的化学反应流程来显示氧浓度如何调节*Cygb*介导NO代谢速率(图2).

在高氧浓度下, *Cygb* ($\text{Fe}^{2+}\text{-O}_2$)浓度增高, 使得有更多的*Cygb* ($\text{Fe}^{2+}\text{-O}_2$)形成并用于氧化NO, 导致NO消耗率增加. 随着 O_2 浓度的降低, NO将更多地结合在*Cygb* (Fe^{2+})上形成*Cygb* ($\text{Fe}^{2+}\text{-NO}$), 从而大大减少了*Cygb* ($\text{Fe}^{2+}\text{-O}_2$)的量. *Cygb* ($\text{Fe}^{2+}\text{-NO}$)不与NO反应, 导致NO消

耗速率的显著降低^[72]. NO代谢的速率还可以通过温度, pH和还原剂的浓度及*Cygb*的浓度来调节^[73]. 基于这些实验结果, Liu等人^[48]假设血管中这种氧依赖的NO代谢很可能是由*Cygb*介导的. 最近的实验结果验证了这一假设.

Li等人^[74]考察了在无氧条件下, *Cygb*介导的亚硝酸盐还原成NO的速率及相关机制. 实验发现, 亚硝酸盐在无氧条件下可从*Cygb* (Fe^{2+})获得一个电子, 生成*Cygb* (Fe^{3+})和NO. 这种亚硝酸盐还原产生NO的过程受pH, 亚硝酸盐浓度和细胞的氧化还原状态的调节. 在组织严重缺氧和酸中毒的缺血条件下, 由*Cygb* (Fe^{2+})介导的亚硝酸盐产生的NO可以作为NO的替代来源来诱导血管舒张.

3 胞红蛋白的表达调控

*Cygb*也可以通过外界条件刺激改变表达, 例如, *Cygb*在大脑中的表达受甲状腺激素状态的影响^[75], 在缺氧的心肌细胞中受钙调磷酸酶活性的影响^[76]. 多数情况下, *Cygb*可以通过某种刺激(如缺氧、氧化应激和纤维化刺激等)使得表达上调, 起到保护细胞的作用^[77,78]. Fordel等人^[79]用双氧水刺激人的SH-SY5Y神经母瘤细胞, 证明了*Cygb*过表达能减少氧化应激引起的细胞死亡. 后来Li等人^[41]也用双氧水刺激神经母瘤细胞, 促使*Cygb*表达上调, 起到保护细胞免受氧化应激损害的作用. 另外Cui等人^[80]用arundic acid诱导

图2 *Cygb*介导的氧依赖的NO代谢分子机制

Cygb的表达, 从而抑制自由基诱导的肝星状细胞的活化, 起到了抗纤维化作用. Lü等人^[81]也证明Cygb有保护活化的肝星状细胞抵抗氧化应激损害的作用. Hodges等人^[82]用实验证明神经元细胞中的Cygb通过抵抗氧化损伤来保护细胞, 并对缺氧缺血再灌注引起的细胞损伤有保护作用. Fordel等人^[83]也证明了Cygb在缺氧缺血条件下表达上调, 可以作为活性氧的清除剂. Schmidt等人^[84]首次详细报道了Cygb mRNA的水平在纤维母细胞和肝星状细胞缺氧时上调. Emara等人^[85]、Chakraborty等人^[86]和Fordel等人^[87]报道Cygb缺氧表达上调出现在体内体外所有组织、正常细胞和癌细胞中, 是防御机制的一部分.

Fordel等人^[88]报道Cygb在海马神经元细胞和鼠的代谢组织中缺氧表达上调的机制与HIF-1 α 紧密相关. HIF-1 α 位于细胞质中, 由氧来调节其稳定性, 在常氧下极易降解, 其活性主要被泛素-蛋白酶体降解途径所控制. 在常氧条件下, 脯氨酸羟化酶(prolylhydroxylase, PHDs)可以使HIF-1 α 的564位和402位脯氨酸残基基化. 泛素-蛋白酶体主要结合在羟基化的HIF-1 α 依赖性降解结构域(oxygen dependent degradation domain, ODD)上, 通过泛素蛋白酶体的降解而降解. 在缺氧条件下, 脯氨酸羟化酶活性被抑制, HIF-1 α 因不能被羟基化而趋于稳定, 与HIF-1 β 亚基结合形成异源二聚体, 异源二聚体和Cygb启动子区域的缺氧反应元件相结合上调Cygb的表达. 这表明Cygb启动子区域包含缺氧反应元件(hypoxia response element, HRE). 后来, Guo等人^[89]用凝胶迁移和染色质免疫沉淀分析确认缺氧诱导因子HIF-1结合了HRE, HRE序列直接涉及Cygb低氧时的转录活性.

John等人同时证明DNA损伤也可以诱导Cygb表达上调. Cygb作为基因损伤应激性蛋白, 与一种关键的细胞损伤因子p53结合, 阻断了其泛素化和随后的降解, 延长了p53的半衰期. p53是通过靶基因p21介导驱使细胞增殖周期停滞, 限制有潜在致癌突变的细胞增殖, 为细胞进行基因修复提供时间^[90]. DNA损伤后, Cygb表达上调, 使得p53及其靶基因p21快速增加并向细胞提示有增殖缺陷. p53通路可识别微小的双链断裂和DNA单链间隙, 此通路一旦被激活便可通过之后的一系列生物学效应而决定细胞的命运, 包括细胞增殖周期的阻滞、细胞衰老、凋亡、DNA修复等. Cygb基因敲除的细胞却不能有效地在G₁期阻止细胞

增殖以响应DNA的损伤. 这为Cygb在肿瘤抑制上提供了新的思路^[91].

4 胞血红蛋白与疾病的关系

4.1 胞血红蛋白与肿瘤的关系

染色体17q是抑癌基因富含区, Cygb基因位于此区, 提示Cygb有潜在的抑癌作用^[59,92]. Shaw等人^[58,93]研究发现, Cygb启动子甲基化能使Cygb基因表达沉默, 而肿瘤细胞中Cygb启动子甲基化明显高于正常细胞. Shivapurkar等人^[46]研究发现, Cygb在肺癌细胞中不表达能增加细胞群落, 在肺癌细胞中过表达能减少细胞群落. Thuy le等人^[61]发现, Cygb基因敲除的小鼠更容易患肿瘤疾病. McDonald等人^[64]报道了癌细胞中Cygb表达的量明显低于正常细胞. 这些说明Cygb具有肿瘤抑制作用, 可用于抗癌类药物的开发. 关于Cygb抗肿瘤的机制尚不清楚, 需要做进一步研究.

4.2 胞血红蛋白与纤维化的关系

纤维化是指组织受损伤时, 由于炎症或氧化应激导致器官功能细胞发生坏死, 组织内细胞外基质异常增多和过度沉积的病理过程. He等人^[55]研究证明, Cygb可以激活线粒体介导途径诱导细胞凋亡起到抑制肝纤维化的作用. Xu等人^[56]报道了Cygb过表达可以抑制自由基诱导的成纤维细胞激活和组织纤维化, 使肝损伤引起的纤维化得到修复, 在控制纤维化中起重要作用. Thuy等人^[65]研究发现, 在肝组织中Cygb基因敲除的小鼠有显著的纤维化. 以上说明Cygb有抗纤维化作用, 对已形成的组织纤维化有修复作用, 可用于抗纤维化药物开发.

4.3 胞血红蛋白与血流和血压的关系

NO是一种血管扩张剂, 也是神经、心血管系统间重要的信息传递因子^[94]. 最近证据表明, Cygb具有NOD的作用, 在存在氧和还原剂时, 能将NO氧化为硝酸根^[95]. Cygb代谢NO的速率与氧浓度有关^[68,71,95]. 在常氧条件下, Cygb代谢NO的速率较快, 而在低氧浓度时Cygb代谢NO的速率相对较慢. 血管壁内的NO浓度是由血管壁上NO的产生速率和NO的代谢速率决定的. 若增加血管中NO的产生速率或减小NO的代谢速率, 血管壁内的NO浓度便会增加. 当体内某个组织缺

氧时, 该组织内血管壁中的NO代谢速率会减慢, NO浓度会随之升高, 导致血管舒张, 血管阻力减小, 血流量增加, 使更多氧可以通过血液带入这个缺氧组织区. 在缺氧条件下, 组织内亚硝酸盐也可还原成NO, 成为一个不依赖于NO合酶的NO生成途径^[96-98]. 在由某种疾病或外在因素引起的严重缺氧条件下, Cygb可以将亚硝酸盐还原为NO, 用于增强血管舒张^[72,75]. Liu等人^[48]最新研究发现, 相对于正常型(WT)小鼠, Cygb缺陷小鼠的血管壁中NO代谢的速率显著降低, 使血管壁内NO的浓度增加, 血管舒张, 血压和血管阻力显著降低. 研究还发现, 下调Cygb的表达可矫正由血管紧张素引起的高血压^[99-102]. 以上研究说明Cygb在体内具有调节血压的作用. 通过改变Cygb在血管壁的表达或者改变Cygb NOD的活性, 可以调节NO在血管壁上的代谢速率, 影响血管壁中的NO浓度, 从而起到调节血压的作用. 因此Cygb可用于开发调节血压类药物, 对心血管类疾病的治疗有重要意义.

5 胞红蛋白的发展前景

由于Cygb分子结构的特殊性、表达分布的广泛性和生物学功能的复杂多样性正引起人们越来越多的关注. 科研人员对Cygb结构、功能及反应机制进行了许多研究, 已发现并证明了Cygb的部分生理作用及相关反应机制, 这些成果为进一步的基础和临床研究提供了新的思路. 虽然Cygb在心血管疾病、肿瘤、内脏器官纤维化以及缺氧缺血细胞保护等方面有着重要作用, 但是从目前对Cygb的研究现状来看, 其所涉及的抗癌、清除活性氧以及其他化学反应机制仍然不是很明确, 有待继续研究^[103].

Cygb过表达可以抵抗肿瘤、保护细胞、清除活性氧、抑制纤维化等. 但Cygb在血管平滑肌中过表达可以引起生物体血压的升高^[48], 如何使Cygb既能发挥它的正常功能, 又不引起血压的增高将是一个新的研究课题.

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Structure, function and the related mechanism of cytoglobin

ZHOU DanLei^{1,2}, LIU XiaoPing² & LUO AiQin¹

1 School of Life Science, Beijing Institute of Technology, Beijing 100081, China;

2 Davis Heart and Lung Research Institute and Division of Cardiovascular Medicine, Department of Internal Medicine, College of Medicine, The Ohio State University, Columbus, OH 43210, USA

Cytoglobin (Cygb) is a hemeprotein belonging to the globin superfamily. It is mainly expressed in fibroblasts, cardiomyocytes and vascular smooth muscle cells. The main function of Cygb is unclear. It has been reported that Cygb is involved in oxygen storage, reactive oxygen species detoxification, collagen synthesis, nitric oxide (NO) metabolism, regulation of gene expression, signal transduction, anti-fibrosis, anticancer, and regulation of blood pressure. In this article, we mainly review its structure characteristics, physiological functions, reaction mechanism, and the relationship of Cygb with some diseases, hoping to provide theoretical basis for future research.

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