

Hsp22 对 SCA3/MJD 转基因果蝇的神经保护作用研究 *

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摘要 为了探讨 Hsp22 在 SCA3/MJD 发病机制中的作用, 选用 GMR-GAL4 和 elav-GAL4 驱动子, 利用经典的 GAL4-UAS 系统, 将含有 78 个 CAG 重复扩增的 ataxin-3 蛋白片段(MJDtr-Q78)分别在果蝇眼睛和神经系统选择性表达, 构建 GMR-GAL4/UAS 和 elav-GAL4/UAS 系统 SCA3/MJD 转基因果蝇模型, 然后利用遗传学方法和热休克反应使 Hsp22 在 SCA3/MJD 转基因果蝇眼睛和神经系统以不同水平过表达。结果表明, Hsp22 过表达显著抑制了 MJDtr-Q78 蛋白的神经毒性, 果蝇眼睛视网膜光感受神经元变性明显缓解, 果蝇存活能力也显著提高。Hsp22 对 SCA3/MJD 具有保护作用, 增强 Hsp22 表达对 SCA3/MJD 可能是一种潜在的治疗方法。

关键词 遗传性脊髓小脑性共济失调 3 型, ataxin-3, Hsp22, 转基因果蝇模型, 神经保护

学科分类号 R74, R394

脊髓小脑性共济失调(spinocerebellar ataxias, SCAs)是一类具有高度临床和遗传异质性的致死、致残率高的神经系统退行性疾病。患病率约 1/10⁶~5/10⁶, 遗传方式多呈常染色体显性遗传。患者多发病于中青年, 临床上以小脑性共济失调为主要特征, 同时还可伴有眼球运动障碍、慢眼活动、视神经萎缩、视网膜色素变性、锥体束征、锥体外系表现、肌萎缩、周围神经病和痴呆等表现。到目前为止, SCA 至少已定位 28 个致病基因位点, 已克隆 18 型, 其中最常见的为 SCA3/MJD 亚型(spinocerebellar ataxia type 3/Machado-Joseph disease, SCA3/MJD), 在中国人群中, 该型几乎占有所有 SCA 患者的 50%^[1~3]。该病发病机制尚未完全阐明, 目前认为, 该病是由于致病基因 *MJD1* 编码区 3'端 CAG 三核苷酸重复序列异常扩增, 导致编码蛋白 ataxin-3 羧基端形成异常扩展的多聚谷氨酰胺(polyglutamine, PolyQ)肽链, 引起 ataxin-3 的错误折叠, 在中枢神经系统小脑等部位的神经元内形成泛素阳性核内包涵体(neuronal intranuclear inclusions, NIIs), 通过蛋白质-蛋白质相互作用产生选择性神经细胞毒性作用而引起的, 故又称为 PolyQ 疾病。

此外, SCA 发病机制还涉及泛素-蛋白酶体途径、转录失调、氧化应激以及生存/凋亡通路等^[4]方面。

在前期研究中我们定位并克隆了腓骨肌萎缩症 2L(Charcot-Marie-Tooth disease type 2L, CMT2L)的致病基因——热休克蛋白 22 基因(heat shock protein 22, *Hsp22*)^[5], 其编码蛋白 Hsp22 属一种小分子热休克蛋白(small heat shock protein, sHsp)家族成员, 具有分子伴侣活性, 在通过促进蛋白质折叠恢复蛋白质正常生理结构和功能、蛋白质转运、抗氧化应激和抗凋亡等方面发挥了重要作用^[6, 7]。

为探讨 Hsp22 对 SCA3/MJD 是否具有保护作用及在 SCA3/MJD 发病机制中的可能作用, 我们

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构建了 GMR-GAL4/UAS 系统和 elav-GAL4/UAS 系统 SCA3/MJD 转基因果蝇模型, 并通过遗传学方法和热休克反应使 Hsp22 以不同水平过表达, 观察 Hsp22 表达对 GMR-GAL4/UAS 系统和 elav-GAL4/UAS 系统 SCA3/MJD 转基因果蝇模型的影响。

1 材料与方法

1.1 材料

基因型 $w[*]; P\{w[+mC]=UAS-Hsp22/MJDtr-Q78\}$ c211.2 的 8150 果蝇品系(简称 UAS-MJDtr-Q78 果蝇), 基因型 $P\{w[+mW.hs]=GawB\}elav[C155]$ 的 458 果蝇品系(简称 elav-GAL4 果蝇)均购自美国 Bloomington 果蝇中心, 基因型 $p\{w[+mC]=longGMR-GAL4\}2/CyO$ 的 8605 果蝇品系(简称 GMR-GAL4 果蝇)由中南大学医学遗传学国家重点实验室提供。

1.2 方法

1.2.1 GMR-GAL4/UAS 系统 SCA3/MJD 转基因果蝇。

a. 构建 GMR-GAL4/UAS 系统 SCA3/MJD 转基因果蝇模型。将 UAS-MJDtr-Q78 处女蝇与 GMR-GAL4 雄性果蝇杂交, 构建基因型为 $w; GMR-GAL4/UAS-MJDtr-Q78$ 的 GMR-GAL4/UAS 系统 SCA3/MJD 转基因果蝇模型。

b. 构建 Hsp22 对 GMR-GAL4/UAS 系统 SCA3/MJD 转基因果蝇干预模型。通过用双平衡系果蝇(基因型 $w; CyO/Sco; TM3/TM6$)对 UAS-MJDtr-Q78 果蝇品系进行平衡, 构建 $w; UAS-MJDtr-Q78; TM3/TM6$ 果蝇; 用双平衡系果蝇对 UAS-Hsp22 果蝇品系(基因型 $+/+; UAS-Hsp22/TM3$)进行平衡, 构建 $CyO/Sco; UAS-Hsp22$ 果蝇; 将 $w; UAS-MJDtr-Q78; TM3/TM6$ 果蝇和 $w; CyO/Sco; UAS-Hsp22$ 果蝇杂交, 筛选出 $w; UAS-MJDtr-Q78/CyO; UAS-Hsp22/TM3$ 果蝇品系。应用能在果蝇眼睛选择性表达的 GMR-GAL4/CyO 果蝇与 UAS-MJDtr-Q78/CyO; TM3/UAS-Hsp22 果蝇杂交, 构建 UAS-MJDtr-Q78/GMR-GAL4; TM3/UAS-Hsp22 (单拷贝)果蝇, 再将其与 UAS-MJDtr-Q78/CyO; TM3/UAS-Hsp22 果蝇杂交, 构建 UAS-MJDtr-Q78/GMR-GAL4; UAS-Hsp22(双拷贝)果蝇。

c. 观察 Hsp22 对 GMR-GAL4/UAS 系统 SCA3/MJD 转基因果蝇模型的影响。将 UAS-MJDtr-Q78/GMR-GAL4; TM3 或 TM6/UAS-Hsp22 果蝇和 UAS-

MJDtr-Q78/GMR-GAL4; UAS-Hsp22 果蝇幼虫置于 37℃ 水浴箱中进行热休克处理 1 h, 每 8 h 处理一次, 共 3 次, 通过电脑体视显微镜观察这两种果蝇热休克前后的复眼结构和色素, 通过电镜分别在放大 250 倍和 1000 倍下观察其单眼结构, 用解剖刀将果蝇眼睛取下后用 2.5% 戊二醛固定, 然后经乙醇脱水、浸透、包埋后制成半薄切片, 用亚甲蓝染色后光镜下观察其复眼内光感受神经元结构。

1.2.2 elav-GAL4/UAS 系统 SCA3/MJD 转基因果蝇。

a. 构建 elav-GAL4/UAS 系统 SCA3/MJD 转基因果蝇。将 UAS-MJDtr-Q78 果蝇处女蝇与 elav-GAL4 果蝇杂交, 构建基因型为 $elav-GAL4/+; UAS-MJDtr-Q78/+$ 的 elav-GAL4/UAS 系统 SCA3/MJD 转基因果蝇模型。

b. 构建 Hsp22 对 elav-GAL4/UAS 系统 SCA3/MJD 转基因果蝇干预模型。应用能在果蝇神经系统选择性表达的 elav-GAL4 果蝇与 UAS-MJDtr-Q78/CyO; TM3/UAS-Hsp22 果蝇杂交, 构建基因型为 $elav-GAL4/+; UAS-MJDtr-Q78/+; UAS-Hsp22/+$ 的 Hsp22 对 elav-GAL4/UAS 系统 SCA3/MJD 转基因果蝇干预模型。

c. 观察 Hsp22 表达对 elav-GAL4/UAS 系统 SCA3/MJD 转基因果蝇模型的影响。将 UAS-MJDtr-Q78 果蝇处女蝇与 elav-GAL4 果蝇杂交后的虫卵以及在 elav-GAL4 果蝇与 UAS-MJDtr-Q78/CyO; TM3/UAS-Hsp22 果蝇杂交后的子代中筛选 $elav-GAL4/+; UAS-MJDtr-Q78/+; UAS-Hsp22/+$ 以及 $elav-GAL4/+; TM3/CyO$ 两种果蝇, 将上述虫卵或幼虫置于 37℃ 水浴箱中进行热休克处理 1 h, 每 8 h 处理一次, 共 3 次。然后计算热休克前后当 $elav-GAL4/+; TM3/CyO$ 羽化成虫数为 100 只时 $elav-GAL4/+; UAS-MJDtr-Q78/+$ 和 $elav-GAL4/+; UAS-MJDtr-Q78/+; UAS-Hsp22/+$ 羽化为成虫的数量。分别取 $elav-GAL4/+; TM3/CyO$ 以及热休克前后的 $elav-GAL4/+; UAS-MJDtr-Q78/+; UAS-Hsp22/+$ 果蝇 100 只, 雌雄分开饲养, 每 2 天更换培养基, 计算寿命。

1.2.3 用实时荧光定量 PCR 检测上述两种经 Hsp22 干预后的 SCA3/MJD 转基因果蝇脑内 Hsp22 mRNA 表达水平。取正常对照果蝇(基因型 $w; GMR-GAL4/+$), GMR-GAL4/UAS 系统 SCA3/MJD 转基因果蝇(基因型 $w; GMR-GAL4/UAS-MJDtr-Q78$), UAS-MJDtr-Q78/GMR-GAL4; TM3/UAS-Hsp22

(单拷贝)果蝇, 热休克的 UAS-MJDtr-Q78/GMR-GAL4; TM3/UAS-Hsp22(单拷贝)果蝇, UAS-MJDtr-Q78/GMR-GAL4; UAS-Hsp22(双拷贝)果蝇, 热休克的 UAS-MJDtr-Q78/GMR-GAL4; UAS-Hsp22(双拷贝)果蝇各 50 只, 将其脑组织切下, 提取 RNA. 取正常对照果蝇, 热休克的正常对照果蝇(基因型 *elav-GAL4*), *elav-GAL4/+*; UAS-MJDtr-Q78/+; UAS-Hsp22/+ 果蝇, 热休克后的 *elav-GAL4/+*; UAS-MJDtr-Q78/+; UAS-Hsp22/+ 果蝇各 50 只, 将其脑组织切下, 提取 RNA. 以 *rp49* 基因为内参, 根据文献[7]合成 Hsp22 和 *rp49* 扩增引物, 采用 SYBR Green 荧光染料在 FTC2000 荧光定量 PCR 仪上进行实时荧光定量 PCR, 对 Hsp22 mRNA 表达水平进行检测, 采用 $2^{-\Delta\Delta C_t}$ 方法计算上述各种品系果蝇脑内 Hsp22 mRNA 表达差异.

2 结 果

2.1 GMR-GAL4/UAS 系统 SCA3/MJD 转基因果蝇

与正常对照野生型果蝇复眼比较(图 1), GMR-

GAL4/UAS 系统 SCA3/MJD 转基因果蝇模型复眼外形不规则、纹路消失、单眼不清楚、正常红色色泽消失, 高电压下完全塌陷、单眼不清楚、刚毛稀少、刚毛短且折断、刚毛排列紊乱, 光感受神经元严重变性. 而在 Hsp22 干预后, 果蝇眼睛呈现明显的好转: UAS-MJDtr-Q78/GMR-GAL4; TM3/UAS-Hsp22(单拷贝)果蝇复眼呈浅红色, 单眼开始成型, 可见到较多光感受神经元成分. 与 SCA3/MJD 转基因果蝇比较, 复眼色素和结构在一定程度上得到恢复, 但大部分果蝇眼睛仍严重破坏, 结构不完整, UAS-MJDtr-Q78/GMR-GAL4; UAS-Hsp22(双拷贝)果蝇则复眼呈亮红色, 色素明显增多, 电镜下呈典型的粗眼, 单眼结构基本成型, 界限清楚, 但刚毛仍然还排列紊乱, 内眼轮廓显现, 光感受神经元成分增多. 进行热休克处理后, UAS-MJDtr-Q78/GMR-GAL4; TM3/UAS-Hsp22(单拷贝)果蝇眼睛较热休克前进一步好转, 果蝇复眼色素明显增多, 呈鲜红色, 电镜下果蝇单眼凸现, 刚毛增多, 视网膜内已经可以见到少量成型的光感受神经元. 而 UAS-MJDtr-Q78/GMR-GAL4;

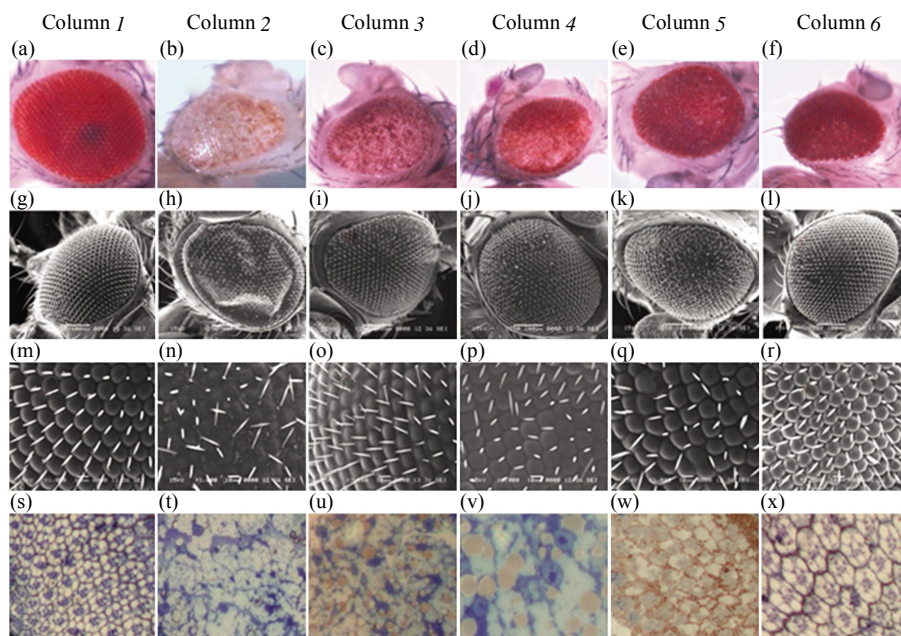


Fig. 1 Overexpression of Hsp22 suppresses polyglutamine-induced degeneration in *Drosophila*

(a)~(f) Dissecting microscopic pictures of the eyes of adult flies. (g)~(l) Scanning electron microscopic pictures of the eyes of adult flies ($\times 250$). (m)~(r) Scanning electron microscopic pictures of the eyes of adult flies ($\times 1000$). (s)~(x) Tangential sections through the eyes of adult flies. Column 1: Control flies expressing only the GMR-GAL4 expression construct. The eye is normal. Genotype of flies is *w*; GMR-GAL4/+ . Column 2: Flies expressing the MJDtr-Q78 protein shows severe retinal degeneration, visualized by loss of photoreceptor rhabdomere specializations and ordered structure of the eye. Genotype of flies is *w*; GMR-GAL4/UAS-MJDtr-Q78. Column 3: Co-expressing only one copy of *Hsp22* gene was mildly effective at suppressing MJDtr-Q78 degeneration, as seen by the slightly less severely disrupted eye compared with the flies expressing alone the MJDtr-Q78 protein. Genotype of flies is *w*; GMR-GAL4/UAS-MJDtr-Q78; TM3/UAS-Hsp22. Column 4: On co-expressing one copy of *Hsp22* gene treated with heat shock restores photoreceptor to the retina and slows degeneration. Genotype of flies is *w*; GMR-GAL4/UAS-MJDtr-Q78; TM3/UAS-Hsp22. Column 5: On co-expressing two copy of *Hsp22* gene, significant retinal structure is now restored such that the characteristic ommatidial pattern of the photoreceptor rhabdomeres is visible within the eye. Genotype of flies is *w*; GMR-GAL4/UAS-MJDtr-Q78; UAS-Hsp22. Column 6: Co-expressing two copy of *Hsp22* gene treated with heat shock almost restored eye structure, obviously suppressing the toxicity of MJDtr-Q78 in photoreceptor neurons, the lattice surrounding the neurons is still abnormal. Genotype of flies is *w*; GMR-GAL4/UAS-MJDtr-Q78; UAS-Hsp22.

UAS-Hsp22(双拷贝)果蝇在热休克后眼睛改善更明显, 红色均匀, 纹路清楚, 几乎接近正常果蝇眼睛; 电镜下果蝇单眼凸现, 刚毛排列整齐, 视网膜光感受细胞完整, 排列规则.

2.2 elav-GAL4/UAS 系统 SCA3/MJD 转基因果蝇
正常野生型果蝇后代见有大量的幼虫出现并羽化成成虫, 而 UAS-MJDtr-Q78 果蝇处女蝇与 elav-GAL4 果蝇杂交后其后代未见成虫出现, elav-GAL4/UAS 系统 SCA3/MJD 转基因果蝇在幼虫期或胚胎期完全致死, 与正常野生型果蝇相比, 其羽化率为正常对照(基因型 elav-GAL4/+; TM3/CyO)的 0%, 而 elav-GAL4/+; UAS-MJDtr-Q78/+; UAS-Hsp22/+ 果蝇幼虫羽化率达到正常对照的 26%, 热休克后 elav-GAL4/+; UAS-MJDtr-Q78/+; UAS-Hsp22/+ 果蝇其幼虫羽化率达到正常对照的 38%, 见图 2. 正常对照野生型果蝇的最长寿命为

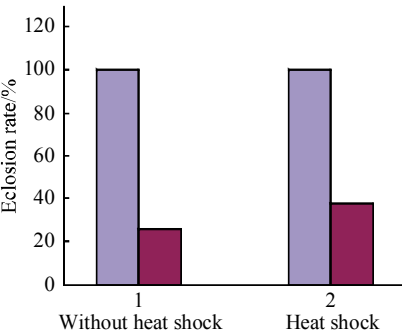


Fig. 2 Difference in rate of eclosion of flies with elav-GAL4

Eclosion rate of flies expressing MJDtr-Q78 with or without heat shock is 0% compared with the control flies, however, eclosion rate of flies expressing MJDtr-Q78 in the presence of *Hsp22* before and after being treated with heat shock increase 26%, 38% respectively. ■: Control; ■: elav-GAL4-MJDtr-Q78-Hsp22; □: elav-GAL4-MJDtr-Q78.

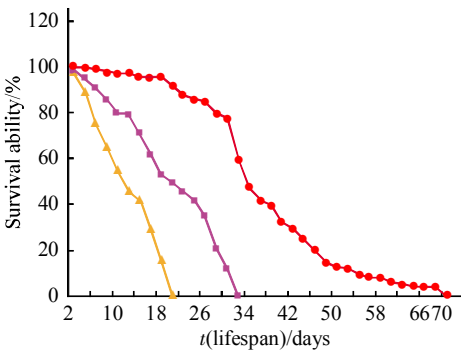


Fig. 3 Increased lifespan in the presence of *Hsp22* expression

Longevity curves of flies expressing *Hsp22* without heat shock (▲—▲), flies expressing *Hsp22* with heat shock (●—●) and of their matched control flies (■—■) are shown. Maximum lifespan are 23, 31, 72 days respectively; mean lifespan are 10, 15, 43 days respectively.

72 天, 平均寿命为 43 天, 而 elav-GAL4/+; UAS-MJDtr-Q78/+; UAS-Hsp22/+ 果蝇热休克前的最长寿命为 23 天, 达到正常对照的 32%, 平均寿命为 10 天, 为正常对照的 23%; 热休克后的 elav-GAL4/+; UAS-MJDtr-Q78/+; UAS-Hsp22/+ 果蝇最长寿命为 31 天, 达到正常对照的 43%, 平均寿命 15 天, 达到正常对照的 35%, 见图 3.

2.3 Hsp22 mRNA 表达水平检测结果

2.3.1 热休克的 GMR-GAL4 系统 SCA3/MJD 转基因果蝇
UAS-MJDtr-Q78/GMR-GAL4; TM3/UAS-Hsp22(单拷贝)果蝇, UAS-MJDtr-Q78/GMR-GAL4; UAS-Hsp22(双拷贝)果蝇, 热休克的 UAS-MJDtr-Q78/GMR-GAL4; TM3/UAS-Hsp22 果蝇, 热休克的 UAS-MJDtr-Q78/GMR-GAL4; UAS-Hsp22(双拷贝)果蝇的 *Hsp22* mRNA 表达水平较 GMR-GAL4 系统 SCA3/MJD 转基因果蝇分别增强了 1.59 倍、1.72 倍、4.11 倍、4.59、4.95 倍, 见图 4.

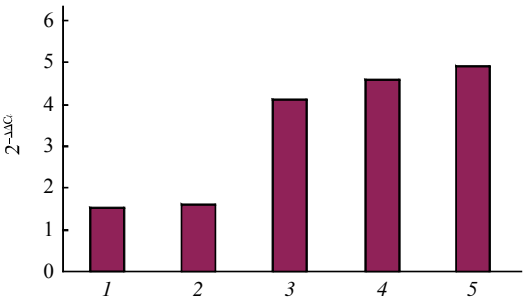


Fig. 4 Difference in the level of expression of *Hsp22* genes among fly lines with GMR-GAL4

RNA was extracted for reverse transcription. Quantitative PCR was performed to determine the inducible mRNA expression levels of *Hsp22* genes. From 1 to 5, 1.59, 1.72, 4.11, 4.59, 4.95-fold increase compared to the control respectively. 1: Fly expressing MJDtr-Q78 protein treated with heat shock. Genotype of flies is w; GMR-GAL4/UAS-MJDtr-Q78; 2: Fly co-expressing MJDtr-Q78 and only one copy of *Hsp22* gene. Genotype of flies is w; GMR-GAL4/UAS-MJDtr-Q78; TM3/UAS-Hsp22; 3: Fly co-expressing expanded polyglutamine protein and only one copy of *Hsp22* gene treated with heat shock; 4: Fly co-expressing expanded polyglutamine protein and two copy of *Hsp22* gene. Genotype of flies is w; GMR-GAL4/UAS-MJDtr-Q78; UAS-Hsp22; 5: Fly co-expressing expanded polyglutamine protein and two copy of *Hsp22* gene treated with heat shock. Control is fly expressing MJDtr-Q78 protein treated without heat shock. The data shows the mean value of 3 independent experiments.

2.3.2 热休克的 elav-GAL4/+; +/CyO; +/TM3 果蝇
elav-GAL4/+; UAS-MJDtr-Q78/+; UAS-Hsp22/+ 果蝇, 热休克的 elav-GAL4/+; UAS-MJDtr-Q78/+; UAS-Hsp22/+ 果蝇的 *Hsp22* mRNA 表达水平较 elav-GAL4/+; +/CyO; +/TM3 果蝇分别增强了 1.2 倍、5 倍、7.6 倍, 见图 5.

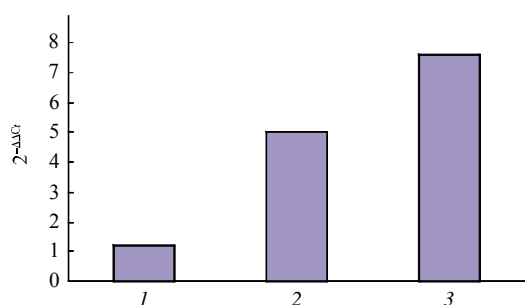


Fig. 5 Difference in the level of expression of *Hsp22* genes among fly lines with *elav-GAL4*

RNA was extracted for reverse transcription. Quantitative PCR was performed to determine the inducible mRNA expression levels of *Hsp22* genes. From 1 to 3, 1.2, 5, 7.6-fold increase compared to the control respectively. 1: Fly expressing only the *elav-GAL4* expression construct treated with heat shock. Genotype of flies is *elav-GAL4/+; +/CyO; +/TM3*; 2: Fly co-expressing expanded polyglutamine protein and *Hsp22* gene. Genotype of flies is *elav-GAL4/+; UAS-MJDtr-Q78/+; UAS-Hsp22/+*; 3: Fly co-expressing expanded polyglutamine protein and *Hsp22* gene treated with heat shock. Control is fly expressing only the *elav-GAL4* expression construct treated without heat shock.

3 讨 论

目前已发现,至少有9种与PolyQ蛋白有关的神经系统遗传病,包括SCA1、SCA2、SCA3、SCA6、SCA7、SCA17、齿状核红核苍白球路易体萎缩症(Dentatorubral-pallidoluysian atrophy, DRPLA)、延脊肌萎缩症(Spinal bulbar muscular atrophy, SBMA)和亨廷顿舞蹈病(Huntington disease, HD)。病理性扩展的PolyQ蛋白具有神经毒性,导致中枢神经系统特定部位神经元的变性和死亡^[8]。PolyQ疾病中神经元的变性和死亡是一种慢性进展过程,利用转基因动物尤其是果蝇模型来模拟并进行干预已经成为此类疾病研究的热点^[9]。目前, PolyQ疾病转基因果蝇模型大部分采用GAL4/UAS系统来表达外源性PolyQ疾病相关基因,其中尤以用GMR-GAL4启动子将PolyQ疾病基因在果蝇眼睛表达的方式最多。众所周知,果蝇眼睛为复眼结构,具有特征性的光感受神经元分布,视网膜的整个发育过程已很清楚,因此通过构建PolyQ疾病转基因果蝇模型并观察转基因后果蝇眼睛结构的动态变化,可以模拟PolyQ疾病并了解PolyQ蛋白对神经元的影响^[10~12]。国外已先后成功建立了SCA1、SCA2、SCA3/MJD、HD、SBMA等多种PolyQ疾病转基因果蝇模型^[13]。

Hsp是分子伴侣家族的重要成员,参与介导蛋白质的正确折叠、聚集、降解以及抵御氧化应激,

主要在热应激条件下诱导产生,其中Hsp22属一种小分子热休克蛋白(small heat shock protein, sHsp)家族成员,在通过促进蛋白质折叠恢复蛋白质正常生理结构和功能方面发挥了重要作用^[14]。同时, Hsp22还是一种线粒体蛋白,线粒体对活性氧簇非常敏感,提高Hsp22的表达水平有助于保护线粒体蛋白防御氧化应激^[15, 16]。另外, Hsp22还参与细胞凋亡、热耐受、免疫调控、抗肿瘤及病毒感染等病理生理过程,进而从多个方面影响着生物体寿命^[17]。

研究发现, SCA3/MJD等PolyQ疾病中存在标志性的病理改变——神经元核内包涵体(NIIs),而在PolyQ疾病患者脑内、转基因细胞和动物模型中,大量的热休克蛋白被募集进入NIIs,并导致细胞内蛋白质折叠,蛋白质聚合物解聚和PolyQ蛋白降解以及应激机制被激活等^[15]。国外研究已证实大分子热休克蛋白如Hsp70、Hsp40等对SCA3/MJD及其他PolyQ疾病动物模型具有明显的保护作用^[5, 18~21]。

为证实小分子热休克蛋白Hsp22也在SCA3/MJD发病机制中起重要作用,本研究利用GAL4/UAS系统,选择GMR-GAL4启动子,使MJDtr-Q78蛋白在果蝇眼睛特异性表达,发现果蝇眼睛视网膜光感受神经元显著变性,眼睛结构几乎完全被破坏。而后将MJDtr-Q78蛋白和Hsp22蛋白在果蝇眼睛共表达,并通过遗传学方法和热休克反应使Hsp22以不同水平表达,观察Hsp22对毒性MJDtr-Q78蛋白所致的果蝇复眼变性的影响,结果发现,在Hsp22的干预下,果蝇眼睛有了明显的好转,且随着Hsp22 mRNA表达水平的提高,果蝇眼睛结构的恢复也相应地更加明显,表明果蝇眼睛的恢复水平与Hsp22 mRNA的表达水平相一致,两者存在剂量-效应关系,进一步说明Hsp22抑制了MJDtr-Q78蛋白的毒性,对GMR-GAL4/UAS系统SCA3/MJD转基因果蝇模型具有明显的神经保护作用。

同时, MJDtr-Q78蛋白在果蝇神经系统表达,选择性导致神经系统明显变性,果蝇发育受阻,成虫完全致死,表明MJDtr-Q78具有明显的神经毒性。而在*elav-GAL4/+; UAS-MJDtr-Q78/+; UAS-Hsp22/+*果蝇中,通过在果蝇神经系统过表达Hsp22发现,果蝇发育受限在很大程度上得到缓解,果蝇羽化能力明显提高,且随着Hsp22 mRNA表达水平的提高,果蝇存活能力也明显提高,两者

存在剂量 - 效应关系, 也进一步说明 Hsp22 抑制了 MJDtr-Q78 蛋白的神经毒性, 对 elav-GAL4/UA 系统 SCA3/MJD 转基因果蝇模型具有明显的神经保护作用。

上述研究证明, Hsp22 对 SCA3/MJD 转基因果蝇模型具有明显的神经保护作用, 推测其机制可能是由于 Hsp22 改善了异常扩增的 PolyQ 蛋白的错误折叠, 增强了线粒体蛋白防御氧化应激的能力, 从而抑制了 PolyQ 蛋白的细胞毒性, 因而保护了 SCA3/MJD 转基因果蝇。

Hsp22 对 SCA3/MJD 转基因果蝇模型的保护作用提示, 增强某些热休克蛋白等分子伴侣活性, 对包括 PolyQ 疾病在内的神经退行性疾病和遗传病或许是一种潜在的治疗方法, 同时在此类疾病研究中应用转基因果蝇模型有助于更好地探讨其发病机制。

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Research on Neuroprotective Role of Hsp22 in SCA3/MJD Transgenic *Drosophila* Models*

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Abstract To confer the influence of Hsp22 on pathogenesis of SCA3/MJD. GMR-GAL4 and elav-GAL4 system SCA3/MJD transgenic *Drosophila* models were constructed by using the promoter GMR-GAL4 and elav-GAL4 which drive target selective gene expression in developing eyes and neurons, respectively. Then, Hsp22 protein was overexpressed in SCA3/MJD transgenic *Drosophila* models at different levels by genetic methods and heat shock reaction. Overexpression of endogen *Drosophila* Hsp22 can notably suppress the neurotoxicity of MJDtr-Q78 protein, and the level of Hsp22 expression was in consistent with rehabilitation for neurodegeneration of *Drosophila* eyes, and extension of *Drosophila* lifespan. It is firstly confirmed that expression of Hsp22 protects the SCA3/MJD from neurodegeneration on *Drosophila* models, which might contribute to a potential therapeutic effect on SCA3/MJD.

Key words SCA3, ataxin-3, Hsp22, transgenic *Drosophila* models, neuroprotection

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