DOI:10.16016/j.1000-5404.2002.07.022
第24卷第7期 第三军医大学学报 Vol. 24, No. 7
802 2002年7月 ACTA ACADEMIAE MEDICINAE MILITARIS TERTIAE Jul. 2002

论著

文章编号:1000-5404(2002)07-0802-03

# 胆道梗阻肝脏部分切除后肝细胞周期素及其依赖性激酶mRNA的表达变化

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提 要:目的 检测胆道梗阻肝脏部分切除术(PH)后肝细胞周期素(cyclin)$D_1$、E及细胞周期素依赖性激酶CDK2、CDK4 mRNA的表达变化。方法 Wistar大鼠随机分为正常70%肝部分切除组(N-PH)、胆道梗阻(BDO)胆流再通(RBF)70%PH组(BDO-PH)及胆道梗阻胆流再通组(BDO-RBF)。RT-PCR法检测肝细胞cyclin $D_1$、cyclin E mRNA及CDK2、CDK4 mRNA表达。结果 BDO-PH后肝细胞cyclin $D_1$ mRNA和CDK4 mRNA、cyclin E mRNA和CDK2 mRNA表达变化时限一致,其表达增长缓慢,表达高峰晚于N-PH组12~24 h,分别于70%PH后24 h及48 h达到高峰,且其高峰表达值亦明显低于N-PH组。结论 胆道梗阻大鼠70%PH后肝细胞cyclin $D_1$ mRNA、cyclin E mRNA及CDK2 mRNA、CDK4 mRNA表达高峰延后,且表达量减少。

关键词:肝再生;胆道梗阻;细胞周期素;细胞周期素依赖性激酶
中图法分类号:R329.28;R394.2;R657.46 文献标识码:A

# Expressions of cyclins mRNAs and CDKs mRNA in hepatocytes after 70% partial hepatectomy in bile duct obstructive rats

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**Abstract**: **Objective** To investigate mRNA expressions of cyclin D1, cyclin E, CDK2 and CDK4 by regenerative hepatocytes after 70% partial hepatectomy (70% PH) in bile duct obstructive rats. **Methods** Wistar rats were divided randomly into N-PH group in which 70% partial hepatectomy were operated, BDO-PH group in which 70% partial hepatectomy were operated after restoration of bile flow (RBF) following 1 week's bile duct obstruction and BDO-RBF group in which only RBF were operated after BDO. Expressions of cyclin D1, cyclin E, CDK2 and CDK4 mRNA in hepatocytes 0, 12, 24, 48, and 72 h and 1 week after 70%PH or RBF were measured with RT-PCR. **Results** Expressions of cyclin D1/CDK4 mRNA and cyclin E/CDK2 mRNA were increased sharply and peaked 12 and 24 h after 70%PH in N-PH group. However, expressions of cyclin D1/CDK4 mRNA and cyclin E/CDK2 mRNA were increased slowly and peaked 24 and 48 h after 70%PH in BDO-PH group, and their peak expressions were less than N-PH group. **Conclusion** Expressions of cyclin D1/CDK4 mRNA and cyclin E/CDK2 mRNA by regenerative hepatocytes after 70%PH are slowly and significantly decreased in bile duct obstructive rats.

**Key words**: liver regeneration; bile duct obstruction; cyclin; CDK

胆道梗阻肝脏部分切除术后肝细胞再生能力减弱,但原因尚未完全清楚。最近研究显示细胞再生过程中最关键的DNA合成和有丝分裂受细胞周期素(Cyclins)及其依赖性激酶(Cyclin-dependent kinase, CDKs)控制,提示细胞周期对细胞再生具有特别重要的调控作用。但cyclins与CDKs在胆道梗阻肝脏部分切除术后肝细胞的表达变化仍不清楚,探讨其表达变化,将可以为临床病肝部分切除术后肝细胞再生调节提供理论依据。

## 1 材料和方法

### 1.1 实验动物分组及模型制作

270只健康雄性Wistar大鼠,体重200~250 g,随机分为正常大鼠70%肝部分切除(Normal partial hepatectomy, N-PH)组,胆道梗阻(Bile duct obstruction, BDO)胆流再通(Restoration of bile flow, RBF)70%肝部分切除(BDO-PH)组及胆道梗阻胆流再通(BDO-RBF)组,每组每时相点动物15只。N-PH组:正常大鼠行肝左叶及中肝叶切除(70%PH)。BDO-PH组:先行胆总管双重结扎并切断制成胆道梗阻模型,1周后行胆流再通及70%PH。BDO-RBF组:胆总管梗阻1周后胆流再通。术后0、12、24、48、72 h及1周,每时相点检测8只大鼠。

### 1.2 肝细胞cyclin $D_1$、cyclin E、CDK2及CDK4 mRNA表达

采用Calley等[1]的方法分离肝细胞。肝细胞总RNA提取,二步法RT-PCR扩增。参照文献[2~4]设计合成cyclin $D_1$、cyclin E、CDK2、CDK4、GAPDH及β-actin引物。引物退火温度为60 ℃,扩增30个循环。扩增产物在2%琼脂糖上电泳。以目的基因mRNA密度值/参照基因mRNA密度值的百分数(%)作为目的基因mRNA的相对表达量。

### 1.3 统计学处理

数据以$\bar{x}\pm s$表示,行$t$检验。

## 2 结果

### 2.1 胆道梗阻肝脏70%PH后肝细胞cyclin $D_1$、cyclin E mRNA表达

BDO-PH后肝细胞cyclin $D_1$ mRNA与cyclin E mRNA表达增长

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收稿日期:2001-03-04;修回日期:2001-09-04

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缓慢, 表达高峰为 PH 后 24～48 h, 晚于 N-PH 组 12～24 h, 且高峰表达值明显低于 N-PH 组。BDO-RBF 后 0 h 肝细胞 cyclins mRNA 表达量与 BDO-PH 组相似, 但术后持续下降至正常水平, 说明两组 cyclins mRNA 表达差异是由 70%PH 所致, 见表 1, 2 及图 1, 2。

表 1　70%PH 后肝细胞 cyclin $D_1$ mRNA 的表达($\bar{x}\pm s$)

Tab 1　Expressions of cyclin D1 mRNA in hepatocytes after 70%PH($\bar{x}\pm s$)

| Group | n | cyclin $D_1$ mRNA/GAPDH mRNA(%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 h | 12 h | 24 h | 48 h | 72 h | 1 weeks |
| N-PH | 8 | 13.74±3.37 | 75.64±9.27*# | 51.50±5.92 | 42.02±5.47 | 30.42±7.46 | 21.54±3.21 |
| BDO-PH | 8 | 20.91±4.61 | 43.70±6.80 | 57.38±7.34 | 46.81±5.96 | 33.04±5.47 | 25.10±5.16 |
| BDO-RBF | 8 | 19.42±5.45 | 17.18±4.34 | 16.02±4.03 | 14.78±4.14 | 12.52±3.59 | 10.36±3.08 |

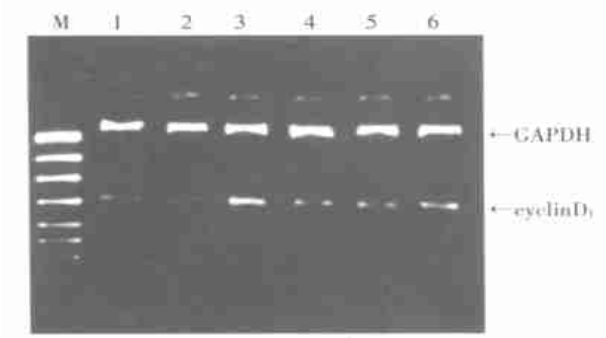
*: $P<0.001$ *vs* N-PH(0 h); #: $P<0.001$ *vs* BDO-PH(12 h, 24 h)

表 2　70%PH 后肝细胞 cyclin E mRNA 表达($\bar{x}\pm s$)

Tab 2　Expression of cyclin E mRNA in hepatocytes after 70%PH($\bar{x}\pm s$)

| Group | n | cyclin $D_1$ mRNA/GAPDH mRNA(%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 h | 12 h | 24 h | 48 h | 72 h | 1 weeks |
| N-PH | 8 | 14.52±3.94 | 33.98±4.10 | 73.88±7.38*# | 53.20±5.84 | 39.10±5.04 | 17.70±3.30 |
| BDO-PH | 8 | 26.52±5.66 | 34.88±5.86 | 44.38±6.42 | 56.84±6.15 | 41.82±6.18 | 29.94±4.01 |
| BDO-RBF | 8 | 24.58±4.62 | 22.96±4.13 | 20.44±4.75 | 18.54±3.73 | 15.36±3.27 | 12.22±2.26 |

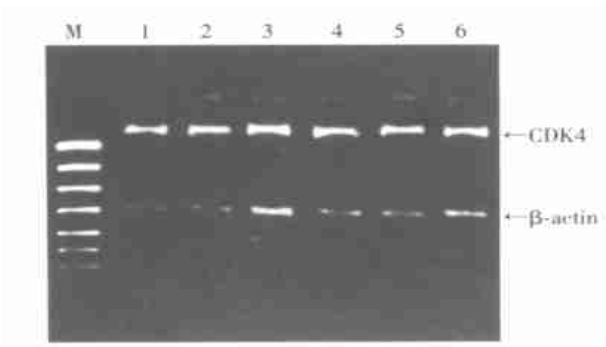
*: $P<0.001$ *vs* N-PH(0 h, 12 h); #: $P<0.001$ *vs* BDO-PH (24 h, 48 h)




M: Marker; 1, 3, 5 and 2, 4, 6: Expressions of cyclin D1 mRNA in the hepatocytes 0, 12, and 24 h after 70%PH in N-PH group and BDO-PH group respectively

图 1　70%PH 后肝细胞 cyclin $D_1$ mRNA 表达(RT-PCR)

**Fig 1　RT-PCR for expression of cyclin D1 mRNA in the hepatocytes after 70%PH**




M: Marker; 1, 3, 5 and 2, 4, 6: Expressions of cyclin E mRNA in hepatocytes 0, 24, and 48 h after 70%PH in N-PH group and BDO-PH group respectively

图 2　70%PH 后肝细胞 cyclin E mRNA 表达(RT-PCR)

**Fig 2　RT-PCR for expression of cyclin E mRNA in the hepatocytes after 70%PH**

## 2.2　胆道梗阻肝脏 70%PH 后肝细胞 CDK2、CDK4 mRNA 表达

BDO-PH 后肝细胞 CDK2 mRNA、CDK4 mRNA 表达增长缓慢, 表达高峰延后至 PH 后 24 h 与 48 h, 晚于 N-PH 组 12～24h, 且高峰表达值明显低于 N-PH 组。BDO-RBF 后 0 h 肝细胞 CDKs mRNA 表达量与 BDO-PH 组相似, 但术后持续下降至正常水平, 说明两组 CDKs mRNA 表达差异是由 70%PH 所致。见表 3、4 及图 3、4。

表 3　70%PH 后肝细胞 CDK2 mRNA 表达($\bar{x}\pm s$)

Tab 3　Expression of CDK2 mRNA in hepatocytes after 70%PH($\bar{x}\pm s$)

| Group | n | CDK2 mRNA/GAPDH mRNA(%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 h | 12 h | 24 h | 48 h | 72 h | 1 weeks |
| N-PH | 8 | 15.21±4.94 | 27.36±5.25 | 74.64±8.29*# | 61.37±6.56 | 34.18±5.57 | 21.27±4.54 |
| BDO-PH | 8 | 23.35±5.48 | 32.39±4.72 | 43.85±6.52 | 56.14±7.43 | 39.42±6.81 | 32.48±6.71 |
| BDO-RBF | 8 | 25.65±6.27 | 23.64±5.32 | 21.12±4.56 | 19.46±4.39 | 15.65±4.73 | 13.82±4.61 |

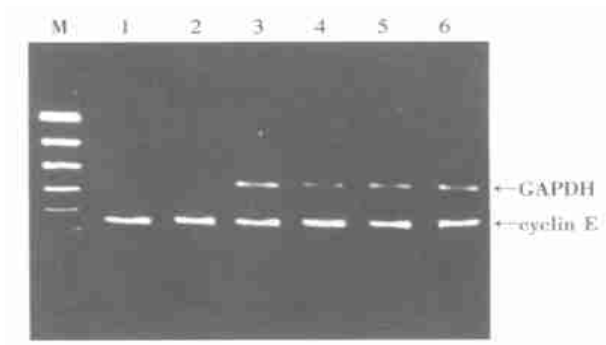
*: $P<0.001$ *vs* N-PH(0 h, 12 h); #: $P<0.001$ *vs* BDO-PH (24 h, 48 h)

表 4　70%PH 后肝细胞 CDK4 mRNA 表达($\bar{x}\pm s$)

Tab 4　Expression of CDK4 mRNA in hepatocytes after 70%PH($\bar{x}\pm s$)

| Group | n | CDK2 mRNA/β-actin mRNA(%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 h | 12 h | 24 h | 48 h | 72 h | 1 weeks |
| N-PH | 8 | 13.63±4.12 | 69.74±9.86*# | 48.57±6.53 | 34.08±6.42 | 21.72±5.37 | 18.46±4.73 |
| BDO-PH | 8 | 19.28±5.74 | 42.85±6.71 | 54.79±7.12 | 45.26±6.83 | 31.48±6.54 | 25.74±5.86 |
| BDO-RBF | 8 | 22.81±6.28 | 20.64±6.39 | 18.74±5.52 | 16.46±4.38 | 14.62±4.23 | 11.92±4.69 |

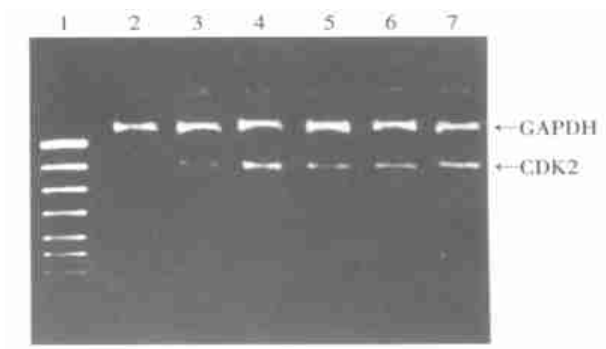
*: $P<0.001$ *vs* N-PH(0 h); #: $P<0.001$ *vs* BDO-PH (12 h, 24 h)




M: Marker; 1, 3, 5 and 2, 4, 6: Expressions of CDK4 mRNA in the hepatocytes 0, 12, and 24 h after 70%PH in N-PH group and BDO-PH group respectively

图 3　70%PH 后肝细胞 CDK4 mRNA 表达(RT-PCR)

**Fig 3　RT-PCR for expression of CDK4 mRNA in the hepatocytes after 70%PH**




M: Marker; 1, 3, 5 and 2, 4, 6: Expressions of CDK2 mRNA in the hepatocytes 0, 24, and 48 h after 70%PH in N-PH group and BDO-PH group respectively

图 4　70%PH 后肝细胞 CDK2 mRNA 表达(RT-PCR)

**Fig 4　RT-PCR for expression of CDK2 mRNA in the hepatocytes after 70%PH**

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## 3 讨论

最近的研究表明控制再生细胞DNA合成和有丝分裂的细胞周期素调节亚基及其依赖性激酶CDKs，具有与HGF、TGF-α、EGF及IL-6等肝细胞生长因子同等重要的促进肝部分切除术后肝细胞再生的作用。

在参与肝再生的众多细胞周期素中，cyclin $D_1$、cyclin E和cyclin B的作用最重要，它们的表达均于肝部分切除术后24 h达到高峰[2,5]。cyclin $D_1$扮演着生长因子感受器的作用，推动细胞由$G_0$期进入$G_1$期，cyclin E在细胞$G_1$/S期过渡时表达增高，推动细胞由$G_1$期进入S期，而cyclin B则主要推动细胞从$G_2$期进入M期，促进细胞分裂。

肝部分切除触发肝细胞进入细胞周期，导致细胞周期激活的信号最终集中到细胞核CDK家族，细胞核内连续的CDK的激活是细胞周期过程的根本。CDKs与调节亚基cyclin相结合后被活化，在细胞周期不同的阶段有特殊的cyclin/CDK复合物被活化，如cyclin D/CDK4复合物在$G_1$中期激活，cyclin E/CDK2复合物为$G_1$/S过渡所必需、cyclin A/CDK2促进DNA合成、cyclin B/CDK1及cyclin A/CDK2使细胞进入有丝分裂期。有研究表明大鼠70%肝部分切除术后12 h肝细胞CDK4与CDK2的活性开始增高，术后24 h其活性最高，其活性的增高是肝细胞核内CDK4、CDK2蛋白表达急剧增加的结果[5]。但文献[6]报道，大鼠70%PH后肝细胞内CDK2、CDK4蛋白分别于术后24 h、48 h达到高峰。

临床上常对并发或轻或重胆道梗阻的肝内胆管多发结石病人行肝叶切除以彻底消除病灶，而胆道梗阻导致肝部分切除术后肝细胞再生能力减弱[7]，但此时肝再生受抑制是否与肝细胞cyclins及CDKs表达异常有关，目前并无研究报道。我们检测了胆道梗阻1周大鼠70%PH后肝细胞cyclin $D_1$、cyclin E mRNA及CDK2、CDK4 mRNA表达变化，试图为临床病肝部分切除术后肝细胞再生调节提供理论依据。

我们在实验中发现，BDO-PH后肝细胞cyclin $D_1$ mRNA和CDK4 mRNA、cyclin E mRNA和CDK2 mRNA的表达变化时限一致，其mRNA表达增加延缓，表达高峰延后于N-PH组12～24 h，分别于BDO-PH后24 h和48 h达到高峰，且高峰表达量亦明显低于N-PH组。由于BDO-PH后肝细胞cyclin $D_1$ mRNA和CDK4 mRNA、cyclin E mRNA和CDK2 mRNA表达增长缓慢与表达量减少，cyclin $D_1$/CDK4及cyclin E/CDK2复合物的形成高峰与复合物的形成量亦可能延后及减少，并最终导致肝细胞$G_1$期延长及进入M期延后，从而延缓肝细胞增殖。由于HGF对cyclin $D_1$的表达起促进作用，BDO-PH组术后肝内HGF mRNA表达减少[8]可能是肝细胞cyclin $D_1$ mRNA表达高峰延缓与表达量降低的原因之一。由于cyclin E的表达是由E2F1转录因子所介导，而E2F1是被cyclin D依赖性激酶所过度磷酸化的pRb激活的，cyclin E的表达减少可能与cyclin $D_1$的减少有关。另外，由于TGF-β可下调cyclins的表达，胆道梗阻肝脏部分切除术后肝细胞cyclins mRNA表达缓慢与减少亦可能是肝内TGF-β持续表达增高所致[9]。

综上所述，胆道梗阻肝脏部分切除术后肝细胞cyclin $D_1$、cyclin E、CDK2及CDK4 mRNA表达缓慢与表达量减少，导致肝细胞进入S期及M期不力，肝细胞DNA合成与分裂均受到阻碍，从而延缓肝细胞增殖，因此，通过调节肝细胞cyclins与CDKs的表达有可能促进病肝部分切除术后肝细胞再生。

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