**分类号：** 单位代码**：10114**

**密** 级： 学 **号：BS2010001**

**Mps1 突变体在染色体不稳定性中的作用机制研究**

**The molecular mechanisms of Mps1 mutants causing chromosome instability**

**研** 究 **生：刘** 静

**指导教师：崔永萍** **赵浩亮 专业名称：外科学**

**研究方向：肿瘤病因学学位类型：科学学位**

**所在学院：第一临床医学院**

**中国 ft西**

**二○一三年五月八日**

目 录

[中英文对照表](#_Toc686306912) 3

**[Abstract](#_Toc686306913)** 6

[第一章 前 言](#_Toc686306914) 7

[第二章 材料与方法](#_Toc686306915) 7

**[2.1](#_Toc686306916)** [材料](#_Toc686306916) 7

**[2.1.1](#_Toc686306917)** [实验所用细胞系及质粒](#_Toc686306917) 7

**[2.1.2](#_Toc686306918)** [主要试剂与配制](#_Toc686306918) 8

**[2.1.3](#_Toc686306919)** [主要仪器设备与耗材](#_Toc686306919) 15

**[2.2](#_Toc686306920)** [方法](#_Toc686306920) 16

**[2.2.1](#_Toc686306921)****[Mps1](#_Toc686306921)**[突变质粒的构建](#_Toc686306921) 16

**[2.2.2](#_Toc686306922)** [细胞培养](#_Toc686306922) 16

**[2.2.3](#_Toc686306923)** [质粒转化和提取](#_Toc686306923) 16

**[2.2.4](#_Toc686306924)** [标准的磷酸钙转染法](#_Toc686306924) 18

**[2.2.5](#_Toc686306925)****[逆转录病毒的产Th](#_Toc686306925)** 18

**[2.2.6](#_Toc686306926)** [用逆转录病毒上清感染](#_Toc686306926)**[Sbcl2](#_Toc686306926)**[细胞和](#_Toc686306926)**[SK-MEL31](#_Toc686306926)**[细胞](#_Toc686306926) 18

**[2.2.7](#_Toc686306927)****[S](#_Toc686306927)**[期细胞同步化](#_Toc686306927) 19

**[2.2.8](#_Toc686306928)** [免疫荧光鉴定检测](#_Toc686306928) 19

**[2.2.9](#_Toc686306929)****[Westren-blot](#_Toc686306929)** 19

**[2.2.10](#_Toc686306930)** [免疫共沉淀](#_Toc686306930) 20

**[2.2.11](#_Toc686306931)****[Mps1](#_Toc686306931)**[激酶活性检测](#_Toc686306931) 21

**[2.2.12](#_Toc686306932)** [免疫组化](#_Toc686306932) 21

**[2.3](#_Toc686306933)** [数据统计分析](#_Toc686306933) 21

[第三章 实验结果](#_Toc686306934) 21

**[3.1](#_Toc686306935)****[Mps1](#_Toc686306935)**[突变体对中心体复制及纺锤体形成的影响](#_Toc686306935) 21

**[3.2](#_Toc686306936)****[Mps1](#_Toc686306936)**[突变体对](#_Toc686306936)**[Mps1](#_Toc686306936)**[蛋白激酶活性的影响](#_Toc686306936) 22

**[3.3](#_Toc686306937)****[Mps1](#_Toc686306937)**[突变体对染色体分离的影响](#_Toc686306937) 22

**[3.4](#_Toc686306938)****[B-RAFV600E](#_Toc686306938)**[/](#_Toc686306938)**[MAPK](#_Toc686306938)**[信号通路与](#_Toc686306938)**[pS281-Mps1](#_Toc686306938)**[在皮肤癌组织中的相关性分析](#_Toc686306938) 23

[第四章 讨 论](#_Toc686306939) 23

[第五章 结 论](#_Toc686306940) 24

[参考文献：](#_Toc686306941) 24

[综 述](#_Toc686306942) 27

[参考文献：](#_Toc686306943) 28

[个人简历](#_Toc686306944) 31

# 中英文对照表

|  |  |  |
| --- | --- | --- |
| **英文缩写** | **英文全称** | **中文译文** |
| Mps1 | The mono-polar spindle 1 | 单级纺锤体蛋白激酶 1 |
| MBP | Myelin basic protein | 髓鞘碱性蛋白 |
| B-RAF | V-raf murine sarcoma viral  Oncogene homolog B1 | V 型鼠肉瘤病毒癌基因同源 |
| KRAS | Kirsten rat sarcoma viral oncogene  homolog | 科尔斯顿鼠肉瘤病毒原癌基因 |
| APC/C | Anaphase promoting complex/cyclosome | 后期促进复合物/周期体 |
| Cdc27 | Cell Division Cycle 27 | 细胞周期相关蛋白 27 |
| MAPK | Mitogen-activated protein kinases | 促分裂素原活化蛋白激酶 |
| ERK | Extracellular regulated protein kinases | 细胞外调节蛋白激酶 |
| HU | Hydroxyurea | 羟基脲 |
| C225 | Cetuximab | 西妥昔单抗 |
| EGFR | Epidermal growth factor receptor | 表皮生长因子受体 |

|  |  |  |
| --- | --- | --- |
| DMEM | Dullbecco's modified Eagle's medium | 达氏改良依氏培养基 |
| DMSO | Dimethyl sulfoxide | 二甲基亚砜 |
| PBS | Phosphate buffered solution | 磷酸盐缓冲溶液 |
| Hepes | 2-[4-(2-Hydroxyethyl)-1-piperazinyl]  Ethanesulfonic acid | 羟乙基哌嗪乙硫磺酸 |
| WT | Wild type | 野生型 |
| siRNA | Short interferenee RNA | 短干扰 RNA |
| LC-MS/MS | Liquid chromatography with  Tandem mass spetrometry | [液相色谱串联质谱](http://www.doc88.com/p-86115934579.html) |
| IP | Co-Immunoprecipitation | 免疫共沉淀 |
| IIF | Indirect immunofluorescence | 间接免疫荧光 |

**Mps1突变体在染色体不稳定性中的作用机制研究****摘 要**

**研究背景：**目前肿瘤的基因研究已取得了令人瞩目的进展，重要的癌基因激活是主要原因之一。RAS/RAF/ERK信号通路涉及了多个癌基因的激活，与恶性肿瘤的发生密切相关。科研人员已在人类许多肿瘤组织中都发现B-RAF基因的突变，如70%的皮肤癌，50%的甲状腺癌，20%的结肠癌等恶性肿瘤中都检测到B-RAF基因的突变。其中大约90%的点突变为T1796A，该点突变导致缬氨酸被谷氨酸所代替(V600E)，称B-RAFV600E。2008年，有研究首次报道了B-RAF基因在有丝分裂期的新作用，该基因突变后可导致人皮肤癌细胞、黑色素细胞和永生化上皮细胞出现纺锤体异常、染色体不稳定性及非整倍体细胞出现。在此基础上课题组前期又鉴定出癌基因B-RAFV600E新的作用底物—纺锤体检测点激酶Mps1，二者在有丝分裂期均定位于着丝粒和中心体处，B-RAFV600E主要通过增强Mps1的稳定性而使Mps1蛋白水平以及激酶活性显著增加，导致纺锤体检测点过度激活。进一步机制研究发现，癌基因B-RAFV600E通过磷酸化修饰纺锤体监测点激酶Mps1-S281、S436、S821，分别影响Mps1激酶的蛋白质稳定性、激酶活性等。前期研究发现pS281-Mps1通过抑制由APC/CCdc20介导的Mps1蛋白泛素-蛋白酶体降解途径而增强其蛋白稳定性，导致过量Mps1在中心体处聚积。那么B-RAFV600E磷酸化修饰Mps1导致Mps1突变体异常稳定，是否影响肿瘤细胞中心体复制、纺锤体检测点功能？这是一个值得探讨的问题。

**研究目的：**阐明Mps1突变体对中心体复制、纺锤体检测点功能的影响，结合前期研究阐述B-RAFV600E导致肿瘤细胞中心体复制、纺锤体检测点功能异常机制，最终明确癌基因B-RAFV600E在肿瘤细胞染色体不稳定性中的分子机制。

**研究方法：**为明确Mps1突变体对中心体复制、纺锤体检测点功能的影响，我们主要从以下几方面进行研究：

(1)采用HU-arrest assay将SK-MEL31细胞阻断于有丝分裂S期，免疫荧光技术分析Mps1突变体对中心体复制的影响。

(2)以MBP作为底物进行激酶活性检测，Mps1突变体对Mps1激酶活性的影响。

(3) Metaphase-spreads analysis分析Mps1突变体对染色体不稳定性的影响。

(4)购买皮肤癌组织芯片(ME207)，以p-ERK（反映B-RAFV600E激酶活性）、p-S281 Mps1抗体做免疫组化分析B-RAFV600E信号通路与pS281-Mps1在皮肤癌组织芯片中的相关性。

**研究结果：（1）**采用HU-arrest assay将SK-MEL31细胞阻断于有丝分裂S期，免疫荧光技术分析表达WT-Mps1、S281E、S436E、S821E、EEE及AAA细胞中中心体数目的变化发现，与表达WT-Mps1、AAA的细胞相比表达S281E、S436E、

EEE的细胞中心体复制异常（n≥3）细胞比例显著增高，依次为55%、30%、70%。

(2)以MBP作为底物，以kinase-dead（KD即不具有激酶活性）作为对照，进行激酶活性检测，WT-Mps1有效磷酸化修饰MBP，而Mps1-KD则失活。S281E和S821E与野生型相比同样具有激酶活性。然而，S281A和S821A的Mps1激酶活性减弱甚微，S436A却显著抑制了Mps1激酶活性，表明pS436-Mps1 对

Mps1激酶活性至关重要。S436E、EEE比WT-Mps1具有更高的激酶活性。

(3)以pBabe-puro-HA-Mps1-EEE逆转录病毒颗粒感染Sbcl2细胞和SK-MEL31细胞，感染效率达90%以上。96h后，进行metaphase-spreads analysis，结果表明，与空载体对照组相比，稳定表达Mps1-EEE的Sbcl2细胞，SK-MEL31细胞染色体分离异常细胞比例显著增高，其中，主要为多极纺锤体表型，其次亦有少数细胞出现lagging-chromosomes。免疫荧光结果显示稳定表达Mps1-EEE 的

Sbcl2细胞，SK-MEL31细胞中染色体数目大于或小于46的细胞比例显著增加，即非整倍体细胞。

(4)从US Biomax, Inc (Rockville, MD)购买皮肤癌组织芯片(ME207)，该芯片包括正常皮肤对照及皮肤癌不同分期组织。以p-ERK（反映B-RAFV600E激酶活性）、p-S281 Mps1抗体做免疫组化分析，结果发现p-MAPK阳性（包括阳性和强阳性）在正常皮肤组织（0%）、恶性黑色素瘤（48%）、转移黑色素瘤（69%）比例依次显著增高（P＜0.001）。同样pS281-Mps1阳性表达（包括阳性和强阳性）在正常皮肤组织（0%）、恶性黑色素瘤（54%）、转移黑色素瘤（91%）比例依次显著增高（P＜0.001）。并且我们发现显示p-MAPK阳性的病例同时显示pS281-Mps1阳性表达，反之亦然。提示二者在皮肤癌组织中显著相关，并与临床分期呈正相关（r＝0.25, P＝0.002, Spearman test）。

**结论：**综合以上结果表明癌基因B-RAFV600E通过磷酸化修饰有丝纺锤体监测点激酶Mps1-S281、S436、S821，分别影响Mps1激酶的蛋白质稳定性、激酶活性等，进而导致中心体过度复制、多极纺锤体出现，在肿瘤细胞染色体不稳定性、非整倍体细胞形成中发挥重要作用。

**关键词：**Mps1； B-RAFV600E； 染色体不稳定性； 中心体过度复制； 多极纺锤体

**The molecular mechanisms of Mps1 mutants causing Chromosome instability**

**Abstract**

**Background** Activating BRAF mutations that deregulate the MAPK pathway commonly occur in cancer. The B-RAF mutation have been found in many human cancers, such as 70% of Melanoma, 50% of thyroid carcinoma, 20% of colon carcinoma. More than 90% of the oncogenic B-RAF mutations occur as V600E. A novel role of the B-RAF gene in mitosis has been implicated in melanoma in 2008,. The B-RAF mutation was sufficient to induce spindle abnormalities and chromosomal instability, resulting in euploidy in human melanocytes or in immortalized human mammary epithelial cells. Then, Mps1 was identified as a new

Target of B-RAFV600E by MS. B-RAFV600E signaling prolongs activation of the spindle

Checkpoint through stabilizing Mps1 levels in melanoma cells. The previous study has found that phosphorylation of Mps1 at residue S281 by BRAFV600E signaling prevents the degradation of Mps1 by the anaphase promoting complex/cyclosome (APC/C), thus allowing the Mps1 protein to accumulate in excess at centrosomes. However, Whether an Mps1 mutant that mimics phosphorylation at S281 is sufficient to trigger centrosome amplification, leading to chromosome instability in human melanoma cells is a question worth exploring.

**Objective:** To detect the effects of Mps1 mutant on centrosome duplication and spin dle checkpoint function. And elucidate the molecular mechanism of oncogene B-RAF V600E results in chromosome instability in melanoma cells.

**Methods:** In order to detect the effects of Mps1 mutant on centrosome duplication and spindle checkpoint function, we applied the following approaches:

(1) HU-arrest assay was used to block SK-MEL31 cells at S phase and immunofluor escence was used to analyze the effect of Mps1 mutant on centrosome duplication.

(2) MBP was used as the substrate for measuring kinase activity to analyze the effect

Of Mps1 mutant on Mps1 kinase activity.

(3) Metaphase-spreads analysis was used to analyze the effect of Mps1 mutant on chro mosome instability.

(4) Purchasing the skin cancer tissue microarray ( ME207) to analyze association bet ween B-RAFV600E signaling pathway and pS281-Mps1 in skin cancer tissue chip.

**Result (1)** To determine whether the observed centrosome amplification could be due to a defect in centrosome duplication, we transfected S-phase arrested cells with the various Mps1 constructs, and assessed whether Mps1 mutants led to centrosome re-duplication. We found that centrosome number was similar to controls in wild type-, S821E-, and AAA-expressing cells. However, excess centrosomes were observed in roughly 55%, 30%, or 70% of S281E-, 436E-, and EEE-expressing cells, respectively. This explicitly demonstrates that mimicking phosphorylation of Mps1 at S281 and/or S436E causes centrosome re-duplication, and suggests that the centrosome amplification observed in Fig. 3A-B occurs through a defect in centrosome duplication.

(2) We tested whether the phosphorylation of Mps1 at S281, S436, and S821 affects Mps1 kinase activity. For comparison, a kinase-dead (KD), was used as a negative control. As expected, Mps1-WT phosphorylated MBP efficiently whereas Mps1-KD was inactive. Mps1 S281E and S821E have kinase activity comparable to wild type. However, while the S281A and S821A cause only modest reductions in kinase activity, S436A kinase activity was noticeably reduced, suggesting that phosphorylation of S436 is important for the kinase activity of Mps1 in vitro. Moreover, S436E and EEE mutants have higher kinase activity as compared with Mps1-WT.

(3) To determine whether the amplified centrosomes and the resultant spindle abnormalities induced by phospho-mimetic mutation of Mps1 give rise to chromosome missegregation, we examined chromosome segregation in abnormalities. In contrast to vector control cells, expression of the Mps1-EEE mutant in either Sbcl2 or SK-MEL-31 cells resulted in a high frequency of chromosome segregation anomalies, including lagging chromosomes and chromosome bridges. It stands to reason that the high incidence of chromosome mis-segregation observed in the Mps1-EEE mutant expressing cells would result in aneuploidy. To test for this directly, chromosome counts on metaphase spreads were done in vector control or Mps1-EEE mutant expressing Sbcl2 or SK-MEL-31 cells. A mode of 46 chromosomes was observed for vector control cells, indicating that most of the cells in culture are diploid. In contrast, ectopic expression of the Mps1-EEE mutant resulted in the absence of a chromosome mode and a wider distribution of chromosome counts. Taken together, we conclude that phospho-mimetic mutation of Mps1 (EEE) induces aneuploidy in human melanoma cells.

*(4)* We evaluated the correlation between the level of S281-phosphorylated Mps1 and the activity of BRAFV600E signaling by IHC using a tissue microarray of clinical samples (ME207). Analysis of this tissue array revealed that the percentage of p-MAPK positive cases (positive plus strong positive) significantly increased from normal skin (0%) to malignant melanomas (48%) and malignant metastases (69%; *P*

*<.*001). Likewise, p-S281 Mps1 immunoreactivity dramatically increased from normal skin (0%) to malignant melanomas (54%) and malignant metastases (91%; *P*

*<.*001). Furthermore, we found that the samples showing strong p-MAPK staining also showed strong p-S281 Mps1 staining while samples with weak p-MAPK staining showed weak p-S281 Mps1 staining, suggesting that p-S281 Mps1 staining positively correlated with p-MAPK in human melanoma tissues (*r* = 0.25, *p* = 0.002, Spearman test).

**Conclusion** Based on our findings, BRAFV600E signaling would also phosphorylate Mps1 at S436, and that S436E has higher kinase activity. The failure to properly

Degrade Mps1 leads to the accumulation of excess Mps1 with high kinase activity in the vicinity of centrosomes, resulting in centrosome amplification, multipolar spindles, and chromosome mis-segregation, which are potential mechanisms for aneuploidy and chromosome instability in cancer.

**Key words:** B-RAFV600E; Mps1; Chromosomal instability; Centrosome re-duplication; Multipolar spindles

# 第一章 前 言

恶性肿瘤的发生发展是多因素参与、多基因变异积累、多阶段发生的复杂的病理过程。其中主要原因之一是重要的癌基因激活。RAS/RAF/ERK信号通路涉及了多个癌基因的激活，与恶性肿瘤的发生密切相关[1-2]。自从2002年Davies[3]等发现恶性黑色素瘤和结直肠癌中存在体细胞B-RAF基因突变以来，科研人员已在多种恶性肿瘤组织中发现B-RAF基因突变，国外人群70%皮肤癌[2]，50%甲状腺癌[4]，20%结肠癌[5]，14-30%卵巢癌[6]，15%肝癌[7]，5%肺癌及乳腺癌中都检测到B-RAF基因突变[8-9]；B-RAF基因在中国人非肢端皮肤黑色素瘤中突变频率高达43.3% [10]，在中国人结直肠癌、甲状腺癌中的突变频率分别约为26%-65%、25-50%[11-12]，是中国人黑色素瘤、结直肠癌、甲状腺癌中最为常见的基因变异之一。

目前，已经有超过70个B-RAF基因的错义突变被证实，其中除了少数( 11%)位于外显子11上的甘氨酸环，如G463、G465、G468等点突变外，大约90%的点突变为T1796A，该点突变导致缬氨酸被谷氨酸所代替(V600E)，称为B-RAFV600E。该基因在体外激酶活性是野生型的500倍，可持续激活RAF-MEK-ERK信号通路，影响多种恶性肿瘤相关基因的表达，包括CyclinD、

VEGF、C-myc、β3-integrin等，导致细胞过度增殖、分化，从而在肿瘤发生发展中发挥重要作用[13-15]。另外，研究报道B-RAF基因与K-RAS基因虽然同处RAS-RAF-MAPK通路，但两者突变具有相互排它性，只有大约1%肿瘤同时存在RAS和B-RAF基因的突变[16]。亦有研究发现，在NIH3T3细胞中B-RAFV600E能够过度活化ERK，诱导细胞增殖、转化，在裸鼠体内产生肿瘤[17]。近年来科学家又发现了B-RAF基因在有丝分裂期的新作用，该基因参与细胞有丝分裂期纺锤体形成、染色体分离、纺锤体检测点功能调控，在维持细胞染色体稳定性中发挥重要功能[18]；癌基因B-RAFV600E可导致纺锤体结构及定位异常、染色体不稳定性等有丝分裂期异常现象，在肿瘤细胞染色体不稳定性及非整倍体产生中发挥重要作用[19-20]。因此，**B-RAF**V600E**被公认为一个非常重要的癌基因。**

目前对B-RAFV600E的研究主要集中在以下几个领域：

1. **B-RAFV600E在各种肿瘤中突变频率与临床分级、耐药性、预后关系。**如上所述科研人员已经在多种肿瘤中发现B-RAF基因突变[2-12]。我国恶性肿瘤中皮肤黑色素瘤B-RAFV600E突变频率高达43.3%(13/30)[10]、结直肠癌约26%-65%[11]、甲状腺癌约25-50%[12]，并且B-RAFV600E突变型与耐药、预后相关，携带B-RAFV600E突变型肿瘤患者对抗EGFR克隆抗体耐药，无进展生存期和总生存率较B-RAF野生型患者明显缩短[21-23]。因此，以癌基因B-RAFV600E为靶点的肿瘤治疗策略成为科学家的研究热点。

2. **针对B-RAFV600E的各种肿瘤治疗策略。**目前一些针对B-RAF激酶特异性抑制剂已被成功应用于临床癌症治疗，例如PLX4302是RAF激酶特异性抑制剂，其在携带B-RAFV600E突变体黑色素瘤患者的临床治疗中显示显著疗效，但同时发现部分病人存在严重的PLX4032耐药性问题，甚至促进了肿瘤的发生发展[24-25]。尽管RAF激酶抑制剂在体外细胞水平的耐药机制研究2010年以来有所突破，目前仍缺乏有效策略，尚需系统深入研究癌基因B-RAFV600E在肿瘤发生发展中的作用机制，从中鉴定新的靶标基因。

3. **B-RAFV600E在肿瘤发Th发展中作用机制的研究。**人们已经在结肠息肉和良性黑色素瘤中发现B-RAF基因的突变，说明该基因突变是肿瘤发生发展过程中的早期事件[26]。以往研究发现B-RAFV600E在G1期阻滞及细胞衰老、凋亡方面发挥关键作用。Green MR等发现癌基因B-RAFV600E通过IGFBP7途径诱导细胞衰老和凋亡发生[27]。Yamashita等报道B-RAFV600E在甲状腺癌中可以激活NF-κB信号通路[28]，并且可能与p53有关[29]。Lee等报道B-RAFV600E与MST1通路发生交互作用，在甲状腺癌的发生发展中起重要作用[30]。近年来，B-RAFV600E基因在有丝分裂期中的作用研究获得新突破。

4. 本课题组前期对B-RAFV600E基因的研究突破。多年来研究者普遍认为，

B-RAF基因通过RAS/RAF/ERK信号通路参与调控细胞生长、分化、凋亡等生

物学事件。2008年Cell Cycle首次报道了B-RAF基因在有丝分裂期的新作用，该基因在有丝分裂期分布于中心体以及着丝粒处，参与细胞有丝分裂期纺锤体形成、染色体分离、纺锤体检测点功能的调控，在维持细胞染色体稳定性中发挥重要功能（本人导师作为第二作者参与此项研究）[18]。在此基础上，课题组受国家自然科学基金（30872932）资助，分别在人皮肤癌细胞、黑色素细胞和永生化上皮细胞中发现，癌基因B-RAFV600E导致约70%细胞出现纺锤体异常，其中36%细胞形成多极纺锤体，32%细胞虽然是两极纺锤体，但其结构或定位出现异常[20]。进一步经质谱分析又鉴定出癌基因B-RAFV600E新的作用底物—纺锤体蛋白激酶Mps1，二者在有丝分裂期均定位于着丝粒和中心体处，B-RAFV600E主要通过增强Mps1的稳定性而使Mps1蛋白水平以及激酶活性显著增加，导致纺锤体检测点过度激活。进一步机制研究发现，癌基因B-RAFV600E通过磷酸化修饰纺锤体监测点激酶Mps1-S281、S436、S821，分别影响Mps1激酶的蛋白质稳定性、着丝粒定位能力等[31]。

Mps1**(Mono polar spindle 1)**是依赖细胞周期调控的蛋白激酶，在有丝分裂

M期具有最大激酶活性[32]。正常情况下，Mps1是一种极其不稳定的蛋白，中心体复制完毕及细胞进入有丝分裂后期，在细胞内经由蛋白酶介导的泛素化途径降解[33]。目前已经发现在某些肿瘤细胞中存在Mps1突变体，该突变体在细胞内无法经由泛素化途径降解，导致Mps1蛋白异常稳定，从而与肿瘤细胞中心体复制异常、纺锤体检测点功能异常密切相关[34]。最新研究我们发现pS281-Mps1通过抑制由APC/CCdc20介导的Mps1蛋白泛素-蛋白酶体降解途径而增强其蛋白稳定性，导致过量Mps1在中心体处聚积。那么B-RAFV600E磷酸化修饰Mps1导致

Mps1突变体异常稳定，是否影响肿瘤细胞中心体复制、纺锤体检测点功能？这是一个值得探讨的问题。

中心体复制及纺锤体检测点功能异常是恶性肿瘤细胞染色体不稳定性的主要原因之一。虽然我们发现癌基因B-RAFV600E通过磷酸化修饰单级纺锤体蛋白激酶Mps1-S281增强Mps1激酶的蛋白质稳定性，进而导致过量Mps1蛋白聚集

于中心体，在肿瘤细胞纺锤体检测点功能异常中发挥关键作用[36]，但其具体的分子机制仍有大量研究工作亟待开展，如：①Mps1突变体对中心体复制及纺锤体形成的影响；②Mps1突变体对Mps1激酶活性的影响。③Mps1突变体对染色体不稳定性的影响；④B-RAFV600E信号通路与pS281-Mps1在皮肤癌组织芯片中的相关性分析。

本课题拟针对以上内容，采用免疫荧光、免疫组化、免疫共沉淀、激酶活性检测等方法在前期研究基础上进一步深入研究B-RAFV600E在肿瘤细胞中心体复制、纺锤体形成及纺锤体检查点中的作用，寻找其在肿瘤细胞染色体不稳定性中的作用机制，有望为阐明癌基因B-RAFV600E在肿瘤发生发展中的分子机制以及针对B-RAFV600E的新抗肿瘤策略提供一些理论依据。

# 第二章 材料与方法

## **2.1** 材料

### **2.1.1** 实验所用细胞系及质粒

(1) PHF36-GFP-Mps1, pECE-GST-Mps1 wild-type and kinase dead plasmids 由

Mark Winey (University of Colorado)馈赠。

(2) PBabe-puro-(HA/GFP) retroviral vectors由Gary W Reuther (H Lee Moffitt Cancer Center)馈赠。

(3) pSUPER-GFP, pSUPER-GFP-Mps1逆转录病毒由Gary W Reuther（H Lee Moffitt Cancer Center）馈赠。

(4)逆转录病毒包装细胞293T、复制缺陷包装病毒细胞系LA由王传贵（华东师范大学生命医学研究所）馈赠。

**部分质粒图谱如下：**

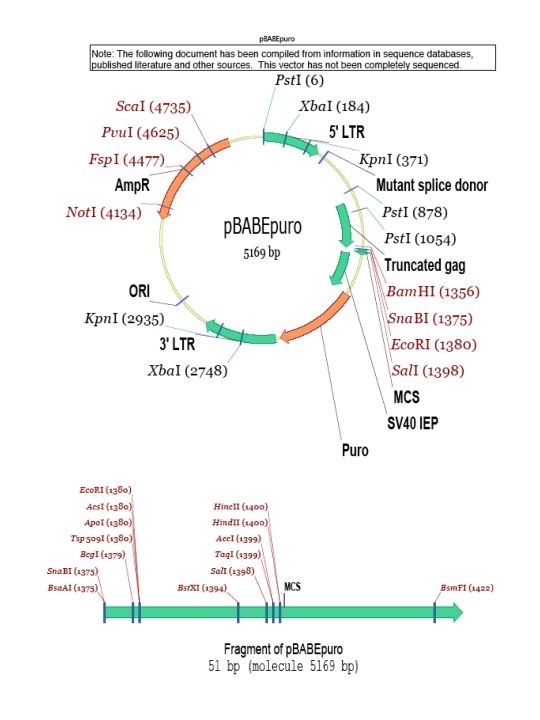


图 1 pBabe-puro质粒图谱

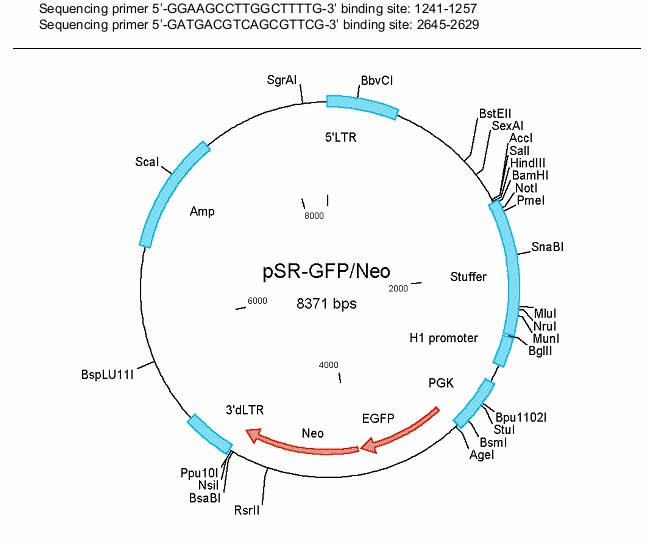


图 2 pSUPER-GFP质粒图谱

**（5）**Sbcl2细胞系来自早期径向生长阶段的原发性黑色素瘤；SK-MEL31细胞系来自晚期垂直生长阶段的恶性黑色素瘤；二者都为野生型BRAF遗传背景，中心体复制、纺锤体结构、纺锤体检查点功能均正常，非整倍体细胞比率≤1%，均由M. Herlyn (Wistar Institute, Philadelphia, PA) 馈赠。

### **2.1.2** 主要试剂与配制

#### **2.1.2.1** 主要试剂

|  |  |
| --- | --- |
| 胎牛血清 | 加拿大 GIBICO 公司 |
| Hepes 缓冲液 | 美国 Sigma 公司 |
| MCDB153 | 美国 Sigma 公司 |
| L15 | 美国 Sigma 公司 |
| 0.25%胰蛋白酶 | 华美生物工程公司 |
| L-谷氨酰胺 | 华美生物工程公司 |
| 青霉素、链霉素 | 华北制药集团公司 |
| 胰岛素 | 美国 Millipore 公司 |
| MEM 丙酮酸钠 | 美国 Invitrogen 公司 |
| 诺考达唑 | 美国 Sigma 公司 |
| 蛋白定量试剂盒 | 美国 Bio-Rad 伯乐公司 |
| 突变试剂盒 | 美国 Stratagene 公司 |
| 突变体引物合成 | 美国 Invitrogen 公司 |
| NaCl | 华美生物工程公司 |

|  |  |
| --- | --- |
| KCl | 华美生物工程公司 |
| CaCl2 | 华美生物工程公司 |
| Na2HPO4·12H2O | 华美生物工程公司 |
| KH2PO4 | 华美生物工程公司 |
| NaHCO3 | 华美生物工程公司 |
| 明胶 | 华美生物工程公司 |
| 非必需氨基酸溶液 | 加拿大 GIBICO 公司 |
| 必需氨基酸溶液 | 加拿大 GIBICO 公司 |
| DMEM/F12 干粉 | 加拿大 GIBICO 公司 |
| 100×的双抗溶液 | 加拿大 GIBICO 公司 |
| 羟基脲(HU) | 美国 Hyclone 公司 |
| DEPC(焦磷酸二乙酯) | 北京鼎国生物有限公司 |
| 皮肤癌组织芯片(ME207) | US Biomax, Inc 公司 |
| 兔抗 Mps1\_hu-pS281-2 | 21 世纪生化公司 |
| 兔抗 MAPK（＃4376） | 21 世纪生化公司 |
| N1 单克隆鼠抗 MPS1 或鼠抗-HA 抗体 | 美国 Sigma 公司 |

|  |  |
| --- | --- |
| α-微管蛋白抗体 | 美国 Sigma 公司 |
| γ-微管蛋白抗体 | 美国 Sigma 公司 |
| 二甲基亚砜(DMSO) | 美国 Sigma 公司 |
| 培养细胞总 RNA 提取试剂盒(离心柱型) (DP430) | 美国 TIANGEN 公司 |
| 感受态细胞 | 美国 TAKARA 公司 |
| 戊二醛 | 美国 Sigma 公司 |
| 吐温-20（Tween-20） | 美国 Sigma 公司 |
| 脱脂奶粉 | 伊利 |
| PVDF 膜 | Bio Rad |
| ECL 荧光底物 | Pierce 公司 |
| 琼脂粉 | 上海生工 |
| 蛋白胨 | 上海生工 |
| 酵母提取物 | 上海生工 |
| 嘌呤霉素 | 上海生工 |

#### **2.1.2.2** 主要溶液的配制

##### **1)** **DMEM/F12**培养基

DMEM/F12干粉1 package

NaHCO3 1.2g

三蒸水补至1L

先用800ml三蒸水溶解DMEM/F12干粉，置磁力搅拌器上并用保鲜膜封口，缓慢搅拌1~2h，使微量营养得到充分溶解，最后称取1.2g NaHCO3加入溶液中，三蒸水定容至1L , PH试纸调节溶液PH值至7.0~7.2，然后滤器过滤除菌(0.22µm滤膜)分装，4℃保存，同时抽样做无菌检验（将过滤后的培养基放入灭菌的离心管中，盖紧盖子，放入37℃细胞培养箱培养4~5天，观察液体是否澄清透亮，出现浑浊则培养基出现污染，需查找原因，配好的培养基暂时不可用，待找到原因后再做处理）。

##### **2)** 无 **Ca2+** 、**Mg2+** **PBS** 缓冲液

NaCl 8g

KCl 0.2g

Na2HPO4·12H2O 2.88g

KH2PO4 0.2g

在800ml三蒸水中，逐项溶解各试剂，置磁力搅拌器上并用保鲜膜封口，缓慢搅拌1~2h，三蒸水定容至1L，PH试纸调节溶液PH值至7.2左右，然后不锈钢滤器过滤除菌(0.22µm滤膜)分装，4℃保存，同时抽样做无菌检验。

##### **3**）细胞固定液配制

25%的戊二醛浓贮液10ml+90ml双蒸水，配成使用浓度为2.5%的戊二醛固

定液，现配现用；4%多聚甲醛固定液购于鼎国试剂公司，4℃避光保存。

|  |  |
| --- | --- |
| **4) 普通细胞冻存液** |  |
| DMEM/F12（含 1%双抗） | 70% |
| FCS | 20% |
| DMSO | 10% |
| **5) 细胞培养液** |  |

293T细胞：DMEM培养基含10%胎牛血清、10mM Hepes、2mM L-谷氨酰胺、1mM MEM丙酮酸钠的

Sbcl2细胞和SK-MEL31细胞：2%肿瘤培养基（MCDB153: L15为4:1、2%

胎牛血清、5mg/ml胰岛素、1mM CaCl2），

**6) 2×HBS：**

NaCl 1.63g

Hepes 1.19g

Na2PO4-2H2O 0.023g

##### **7)** **Kinase Buffer**

Tris-HCL 50mM pH 7.5,

MgCl2, 10mM

Dithiothreitol, 0.5mM

β-glycerophosphate 10mM,

NaVO4 100µM

##### **8)** **Western-blot**液体配制

**A: 细胞裂解液（PLC Lysis Buffer）**

|  |  |  |
| --- | --- | --- |
| 名称 | 终浓度 | 100ml |
| 1M Hepes(pH7.5) | 50mM | 5ml |
| 5M NaCl | 150mM | 3ml |
| Glycerol | 10% | 10ml |
| 50MM MgCl2 | 1.5mM | 3ml |
| Triton×100 | 1% | 1ml |
| 0.5M EDTA(pH8.0) | 1mM | 200μl |
| 0.1M NaPPi | 10mM | 10ml |
| 0.5M NaF | 10mM | 2ml |

每次提取蛋白前加入1％蛋白酶抑制剂。

**B: SDS-PAGE 胶电泳液的配制：**

**①**配制100ml 30%丙烯酰胺：丙烯酰胺29g，甲叉双丙烯酰胺1g，用60ml

ddH2O溶解混匀定容至100ml，4℃保存；

**②**配制100ml 1.5Mol/l PH8.8 Tris-Hcl: Tris 18.17g, 80ml ddH2O溶解，用

HCl调节PH值至8.8定容至100ml；

**③**配制1.0mol/l PH6.8Tris-Hcl: Tris 12.114g, 80ml ddH2O溶解，用HCl

调节PH值至6.8定容至100ml；

**④**配制100ml 10%SDS溶液：SDS 10g, 100ml ddH2O溶解混匀；

**⑤**10%过硫酸胺：过硫酸胺10g, 100ml ddH2O溶解混匀4℃保存；

**⑥**2×SDS凝胶加样缓冲液：将100mmol/L Tirs-Hcl PH6.8, 200mmol/l二硫苏糖醇（DTT），4%电泳级SDS, 0.2% 溴酚蓝，20% 甘油溶解混匀；

**⑦**1mol/l DTT: 20ml 0.01mol/l乙酸钠PH5.2, 3.09g DTT，溶解混匀、过滤除菌，-20℃保存；

**⑧**100ml脱色液：30ml甲醇，10ml乙酸，60ml ddH2O混匀；

**⑨**180ml染色液：90ml甲醇，90ml ddH2O, 10ml乙酸，0.25g考马斯亮蓝R250混匀；

**⑩**Tris-甘氨酸电泳缓冲液（5×储存液）1000ml: 15.1g Tirs碱，94g甘氨酸，10% SDS 50ml, ddH2O溶解混匀、定容至1000ml。

**C：凝胶的配制：**

一般我们需要的浓度为12%。

**①**将玻璃板安装固定好切误把玻璃弄坏；

**②**进行分离胶的配制；

**③**配制Tris-甘氨酸SDS聚丙烯酰胺凝胶电泳分离胶所用溶液：

配制5ml 12%的凝胶：ddH2O 1.6ml , 30%丙烯酰胺2.0ml , 1mol/lTris (PH8.8) 1.3ml, 10%SDS 0.05ml，10%过硫酸铵0.05ml, TEMED 0.004ml；

**④**加入TEMED后立即混匀内容物倒入到固定好的玻璃板中，同时留出灌注浓缩胶所需空间（梳子的齿长再加0.5cm）再在胶液面上小心注入一层水（约2—3mm高）以排除气泡和阻止氧气进入凝胶溶液；

**⑤**分离胶聚合完全后（约15分钟）倾出覆盖水层，再用滤纸吸经残留水；

**⑥**制备浓缩胶：配2ml 的浓缩胶，则需要加水1.4ml，加30%丙烯酰胺

0.33ml，加按下表给出的数据，在另一小烧杯中配制一定体积及一定浓度的丙烯酰胺溶液，加1mol/lTris(PH6.8) 0.25ml, 10%SDS 0.02ml，10%过硫酸铵0.02ml, TEMED 0.004ml。如果配其他体积的浓缩胶则按相应倍数改变。入TEMED后应立即快速旋动混合物倒入到玻璃板中；

**⑦**胶倒入之后将干净的梳子插入到浓缩胶内，注意不要有气泡混入，将凝胶置于室温；

**⑧**浓缩胶聚合后即可进行样品的处理。

**D：转印缓冲液**：

48mmol/L Tris 390mmol/L甘氨酸0.1% SDS 20%甲醇加水定容至1000ml。

##### **9**）菌液培养配方

**A: LB液体培养基：**

称取Luria-Bertani(25.0g/L) 2.5g，溶于100ml一蒸水，不断搅拌；用NaOH调节PH至7.0，加压包扎或锡箔纸包扎；121.3℃高压灭菌30分钟；待降至室温时分装于离心管，4℃保存，一个月内使用。

**B: LB耐药液体培养基：**

称取Luria-Bertani(25.0g/L) 2.5g，溶于100ml一蒸水，不断搅拌；用NaOH调节PH至7.0，加压包扎或锡箔纸包扎；121.3℃高压灭菌30分钟；待温度降至60℃时，加入0.1ml Ampicillin（100mg/ml）或50ulKanamycin

（100mg/ml），均匀混合；分装于离心管，4℃保存，一个月内使用。抗生素终浓度Amp 100ug/ml, Kan 50ug/ml。

**C: LB平板培养基：**

称取Luria-Bertani(25.0g/L) 2.5g，Agar 1.5g；溶于100ml一蒸水，不断搅拌；用NaOH调节PH至7.0，加压包扎或锡箔纸包扎；121.3℃高压灭菌30分钟；待温度降至60℃时，加入0.1ml Ampic-illin（100mg/ml）或50ulKanamycin（100mg/ml），充分摇匀；以10ml 分别铺至平板（30-35ml培养基/90mm培养皿）；培养基倒入培养皿后，敞开盖子，在紫外下照10-15分钟；闭合盖子，封口膜封边，并倒置放于4℃保存，一个月内使用。

### **2.1.3** 主要仪器设备与耗材

#### **2.1.3.1** 主要仪器设备

|  |  |
| --- | --- |
| HS-1300-U 型水平单向流工作台 | 苏州苏净集团 |
| NU-2500 型 CO2 培养箱 | 美国 Nuaire 公司 |
| 研究级倒置相差 DIC 显微成像系统 | 日本 Olympus 公司 |
| 5840R 型高速大容量冷冻离心机 | 德国 Eppendorf 公司 |
| Bio-Rad550 型酶标仪 | 日本 Bio-Rad 公司 |
| JB-2 型恒温磁力搅拌器 | 上海雷磁仪器厂 |
| 超低温冰箱 | Thermo scientific |
| CS101-2AB 电热鼓风干燥箱 | 重庆试验设备厂 |
| HZS-H 型超级恒温水浴振荡器 | 哈尔滨东联电子技术开发有限公司 |
| SP200 型高压灭菌锅 | 日本 YAMATO 公司 |

|  |  |
| --- | --- |
| X 光胶片自动洗片机 | 泰兴中天 |
| 超纯水系统 | 美国 Milli |
| 台式 PH 计 310P-03 | 美国 |
| 垂直电泳系统 Mini-PROTEAN | 美国 |
| 转印系统 Critevion | 美国 |
| 水平电泳系统 Sub-cell | 美国 |
| GDS-8000PC 凝胶成像系统 | 美国 UVP 公司 |
| Eppendorf Biophotometer 分光光度仪 | 德国 Hamburg 公司 |

#### **2.1.3. 2** 主要耗材

1.5ml EP管、15ml、50ml离心管、6孔板、24孔板、96孔板、25cm2培养瓶、10cm培养皿、3ml细胞无菌冻存管：美国Corning公司；0.22µm一次性滤器：Millipore公司。

## **2.2** 方法

### **2.2.1** **Mps1**突变质粒的构建

采用QuikChange®Site-Directed Mutagenesis Kit（Stratagene），构建突变质粒所需引物序列如图2A所示，由Invitrogen公司合成。具体方法详见前期课题组已发表文献（Cui. et al 2010）。分别将所鉴定的三个B-RAFV600E特异的Mps1磷酸化位点（S281、S436、S821）突变为A和E。同时构建Mps1-D-Box-mut、Mps1-WT作为对照组。所有突变体均已经过测序鉴定。



**图1** **A：Mps1突变质粒的构建所需引物序列B：Mps1突变体构建示意图**

### **2.2.2** 细胞培养

293T细胞培养在含10%胎牛血清、10mM Hepes、2mM L-谷氨酰胺、1mM

MEM丙酮酸钠的DMEM培养基中，Sbcl2细胞和SK-MEL采用2%肿瘤培养基

（MCDB153: L15为4:1、2%胎牛血清、5mg/ml胰岛素、1mM CaCl2），待细胞融合度达70%-80%时，以0.25%的胰蛋白酶消化传代，于37℃、5%CO2培养箱中培养。

### **2.2.3** 质粒转化和提取

**A：质粒转化**

将测序鉴定成功的pEF36-HA-Mps1-WT及相应突变体（S281E、S436E、

S821E、EEE、AAA）通过热激法转化入大肠杆菌中。具体步骤如下：

1）自-80℃冰箱中取出感受态大肠杆菌，插入温水中溶解约15min，轻轻混匀，吸取30μl移入1.5mlEp管中，并立刻将剩余细菌放回-80℃冰箱中。

2）加入5μl质粒DNA，轻轻混匀，静置冰上30min。

3）于42℃水浴中热休克细菌45s，迅速移入湿冰中，静置2min。

4）每管中加入500μl LB培养基（不含Amp），混匀，将液体转移至小试管中，封口膜封好，37℃摇床上，225rpm摇晃培养1h。

5）取50μl已转化的感受态细胞滴到含Amp50μg/ml的LB琼脂培养基上，用弯头玻棒（用前需用酒精灯插试，再烧灼，待冷却后使用），轻轻将转化细胞涂匀在琼脂平板表面。

6）将平板略微打开，倒置于室温15min，存放于37℃培养箱中过夜。

##### 7） 次日，检查各培养皿中是否出现菌落。

##### 8） 结果：培养皿中可见散在菌落，转化成功。

##### 9） 挑取单克隆菌落，接种于3ml（Amp）LB培养液，摇菌过夜。

**B：质粒提取**

按照Qia prep spin Mini prep kit（250）试剂盒说明进行：

##### 1)将1.5ml细菌培养液于1.5mlEp管中，5000rpm，离心3min，弃上清，收集菌落。

##### 2） 加入250μl Buffer P1重悬，细菌沉淀，震荡混匀。

##### 3) 加入250μl Buffer P2重悬，通过颠倒4-6次试管混匀。

##### 4) 加入350μl Buffer N3重悬，通过翻转4-6次试管混匀。

##### 5) 3000rpm，离心10min。

##### 6) 把上清放进Qia prep Spin柱。

##### **7）** 13000rpm，离心30-60s，弃漂洗液。

##### 8) 洗柱子，加入0.75ml Buffer PE 13000rpm，离心30s。

##### 9) 弃漂洗液，13000rpm，离心1min。

10)室温静置5min。

11）洗脱DNA：移入新的1.5mlEp管中，加30μl Buffer EB到每个离心柱的中心，室温静置1min。

12) 13000rpm，离心1min。

##### 13） 琼脂糖凝胶电泳检测质粒提取效果。

##### 14） 用Thermo公司NANODROP 2000C核酸蛋白分析仪测定质粒的浓度和纯度。

##### 15） 置于4℃保存。

### **2.2.4** 标准的磷酸钙转染法

将pEF36-HA-Mps1-WT及相应突变体（S281E、S436E、S821E、EEE、AAA）转染293T细胞。具体步骤如下：

##### （1) 传代细胞准备。包装细胞在转染前24h传代，待细胞密度达50%～60%

满底时即可进行转染。加入沉淀前3～4h，用9ml完全培养液培养细胞。

##### (2) DNA沉淀液的准备。首先将质粒DNA用乙醇沉淀（10～50μg/10cm平板），

空气中晾干沉淀，将DNA沉淀重悬于无菌水中，加50μL2.5mol/L CaCl2。

##### （3)用巴斯德吸管在500μL.2×HeBS中逐滴加入DNA-CaCl2溶液，同时用另一吸管吹打溶液，直至DNA-CaCl2溶液滴完，整个过程需缓慢进行，至少需持续1～2min。

(4)静置30min，出现细小颗粒沉淀。

(5)将沉淀逐滴均匀加入10cm平板中，轻轻晃动。

(6)在标准生长条件下培养细胞4～16h.除去培养液，用5ml.1×HeBS洗细胞

2次，加入10ml完全培养液培养细胞。

(7)加入完全培养基24-48h后，收集细胞上清，用0.22μm滤器过滤，分入目的细胞进行感染培养。

### **2.2.5** **逆转录病毒的产Th**

将逆转录病毒空载体pBabe-puro-HA 和pBabe-puro-HA-Mps1-EEE 导入

293T细胞，产生逆转录病毒颗粒，具体方法如下：

(1)在转染前24 h，在铺有4ml的293生长培养基的60mm培养皿中接种包装细胞293T细胞，接种密度大约2.5x106。

(2)转染前移去培养基，并加入新鲜的培养基。

(3)在双蒸水中加入6～10μg质粒DNA，形成鸡尾酒感染混合液，终体积为

438μl. 再加入62μl的2mol/L CaCl2溶液。

(4)加入500μl的2×HBS(pH 7.05)，晃动管子或用移液管吹打均匀，立即将溶液加到细胞上，并轻旋平皿使混合均匀。然后，将细胞放回培养箱中培养。

(5)转染36 h后，移去培养基，并温和地加入3ml新鲜的293T培养基。

(6)大约在转染48 h后，收获逆转录病毒上清。轻轻地移出上清，用0.22μm

的滤膜过滤，或在4℃以500g离心5min以除去活细胞。

(7)如果在1～2 h内使用逆转录病毒上清，将其放置在冰上。若时间间隔较长时，置于干冰上冰冻，而后转到-70℃贮存。

(8)融化冰冻的上清，在37℃下加温片刻，立即使用。

### **2.2.6** 用逆转录病毒上清感染**Sbcl2**细胞和**SK-MEL31**细胞

用逆转录病毒pBabe-puro-HA 和pBabe-puro-HA-Mps1-EEE 上清感染

Sbcl2细胞和SK-MEL31细胞，具体如下：

##### （1) 在感染前12～18h，将5x105的Sbcl2细胞和SK-MEL细胞铺于100mm的平皿中。

##### （2) 准备3ml 的包含逆转录病毒上清、4μg/ml 的polybrene，和Sbcl2 细胞和

SK-MEL细胞培养基的感染混合剂。

##### （3) 从铺有Sbcl2细胞和SK-MEL细胞的平皿上移去培养基，并加上感染混合剂。

##### （4) 将平皿放回培养箱中培养至少3 h。

##### （5) 向平皿上分别相应加入7mlSbcl2细胞和SK-MEL细胞培养基。

##### （6) 转导细胞在感染后36～48 h可以进行检测。

### **2.2.7** **S**期细胞同步化

培养293T、Sbcl2细胞、SK-MEL31细胞至对数生长期、传代、接种入6孔培养皿，浓度为5×104/ ml，加入HU至其终浓度为1mmol/ L，在上述条件下培养24h。弃去培养液，以PBS液清洗细胞后加入新鲜培养液，然后继续培养至感染前。

### **2.2.8** 免疫荧光鉴定检测

以anti-γ和anti-αtublin抗体进行免疫荧光技术分析中心体数目异常及多极

纺锤体形成（n≥3）细胞百分比。按照试剂盒步骤，主要步骤如下：

##### （1) 0.02M的PBS清洗细胞2次，每次5min；

##### （2) 用冷4%多聚甲醛溶液室温20min;

##### （3) 用0.02M的PBS清洗细胞3次，每次10min；

(4) 30%H2O21份＋纯甲醇溶液50份制作混合管，每孔加入500µl，室温放置30min，以灭活内源性过氧化酶；

(5)用0.02M的PBS清洗细胞3次，每次10min；

(6)滴加200µl的5%BSA封闭液，室温放置20min~30min；

(7)吸走封闭液，每孔加入200µl 适当稀释的一抗（一抗用封闭液稀释比例为

（1:100），在4℃湿盒中孵育过夜；

(8)吸走一抗，用0.02M的PBS清洗细胞3次，每次10min；

(9)滴加适量二抗，37℃培养箱中孵育20min；

(10)用0.02M的PBS清洗细胞3次，每次10min；

(11)将免疫荧光结果在荧光显微镜下观察和拍照。

### **2.2.9** **Westren-blot**

Western bloting检测Mps1-WT及相应突变体蛋白的表达情况。具体步骤如

下：

##### **（1)** 样品的处理：

根据蛋白提供的浓度计算上样量

**①**依次取样上样量为10ug；

**②**加入到20ul 2×SDS凝胶加样缓冲液中；

**③**沸水中煮沸8min使蛋白变性；

**④**10000rpm离心5min.

##### **（2)** 样品处理好后即可进行上样：

**①**先将梳子小心的移出，把凝胶固定在电泳装置上；

**②**内外槽各加入1×Tris-甘氨酸电泳缓冲液；

**③**取样品用加样器向孔内加入样品，加样量通常在10~20µl。准备电泳；

**④**将电泳装置与电源连接，接通电源凝胶上胶电压为60伏，当染料前沿进入分离胶后（跑平即可），把电压提高到120V。继续电泳直至溴酚蓝到达分离胶底部上方约0.5cm，然后关闭电源；

**⑤**从电泳装置上卸下玻璃板，用刮勺撬开玻璃板，在凝胶上部一角处切去一角标注加样顺序。

##### **（3)** 免疫印迹

**①**将电泳结束后的胶片放入转印缓冲液中，将转印膜也放入转印缓冲液中浸润；

**②**进行转膜，膜在正极，胶在负极，分别用滤纸3张/面压住胶和膜。转移

时电压100伏，转移时间1小时，电转移时会产热，固应在转移装置的外面加入冰块进行除热；

**③**转移结束后，卸下电泳装置，把硝酸纤维素转移膜放入盛有10%脱脂奶粉或10%BSA的封闭液中，封闭2小时或过夜；

**④**封闭完毕后用PBST洗膜5次，每次间隔5分钟，加一抗。反应1小时，

洗膜5次每次间隔5分钟。（一抗1: 500）

**⑤**加辣根过氧化物酶标记二抗，按说明书要求的稀释比例加入，反应1 小

时。洗膜5次每次间隔5分钟。（二抗1: 1000 ）；

**⑥**取ECL荧光底物（Pierce公司，货号：37071）A液和B液各1ml，混匀后，将PVDF膜浸泡其中，室温孵育3-5分钟后，用滤纸将PVDF膜表面液体擦干，并将其置于暗室，取医用X光底片覆盖，曝光1分钟后，依次显影、定影即可。

### **2.2.10** 免疫共沉淀

将转染成功的293T细胞包括pEF36-HA-Mps1-WT及相应突变体（S281E、

S436E、S821E、EEE、AAA），KD进行免疫共沉淀。具体步骤如下：

(1)收获细胞，加入适量细胞裂解缓冲液（含蛋白酶抑制剂），冰上裂解30min，

细胞裂解液于4℃，最大转速离心30 min后取上清。

(2)取少量裂解液以备Western bloting分析，剩余裂解液加1μg相应的抗体加入到细胞裂解液，4℃缓慢摇晃孵育过夜。

(3)取10μl G-SepharoseTM结合anti-GFP抗体的琼脂糖珠，复合物分别用buffer I (1M NaCl, 1% NP-40, 50mM Tris-HCl PH 8.0), buffer II (200mM NaCl, 0.1% NP-40, 50mM Tris-HCl PH 8.0), buffer III (150mM NaCl, 0.01% NP-40, 50mM Tris-HCl PH 8.0)冲洗3次，每次3,000 rpm离心3 min。

(4)将预处理过的10μl G-SepharoseTM结合anti-GFP抗体的琼脂糖珠加入到和anti-HA（anti-Cdc27）抗体孵育过夜的细胞裂解液中4℃缓慢摇晃孵育2-4h，使抗体与G-SepharoseTM结合anti-GFP抗体琼脂糖珠偶连。

(5)免疫沉淀反应后，在4℃以3, 000 rpm速度离心3 min，将琼脂糖珠离心至管底；将上清小心吸去，琼脂糖珠用1ml裂解缓冲液洗3-4次；最后加入15μl的2×SDS上样缓冲液，沸水煮5min。

(6) SDS-PAGE, Western bloting分析。

### **2.2.11** **Mps1**激酶活性检测

Mps1免疫共沉淀复合物在kinase buffer中孵育后，加入2µCi of [γ32P] ATP和0.5 mg/ml MBP进行激酶活性检测。加入gel sample buffer停滞激酶反应，95℃高温加热5min. SDS-PAGE检测蛋白表达，MBP中加入32P进行显影。

### **2.2.12** 免疫组化

皮肤癌组织芯片购自US Biomax, Inc (Rockville, MD). ME207 ([http: //www. biomax. us/tissue-arrays/Melanoma/ME207](http://www.biomax.us/tissue-arrays/Melanoma/ME207)), 高密度芯片(69cases/207

cores），包括30例多发原发性黑色素瘤/90点、30例转移黑色素瘤/90点及9例正常正常皮肤组织/27点。

按照试剂盒步骤，主要步骤如下：

##### （1) 0.02M的PBS清洗细胞2次，每次5min；

##### （2) 用冷4%多聚甲醛溶液室温20min;

##### （3) 用0.02M的PBS清洗细胞3次，每次10min；

(4) 30%H2O21份＋纯甲醇溶液50份制作混合管，每孔加入500µl，室温放置30min，以灭活内源性过氧化酶；

(5)用0.02M的PBS清洗细胞3次，每次10min；

##### （6) 滴加200µl的5%ft羊血清封闭液，室温放置30min~60min；

(7)吸走封闭液，每孔加入200µl适当稀释的一抗，在4℃湿盒中孵育过夜；(一抗DESMIN用封闭液稀释比例为1: 100)；

(8)吸走一抗，用0.02M的PBS清洗细胞3次，每次10min；

##### （9) 滴加适量生物素化ft羊抗兔IgG，37℃培养箱中孵育20min；

(10)用0.02M的PBS清洗细胞3次，每次10min；

##### （11) 滴加适量SABC试剂，37℃培养箱中孵育20min;

##### （12) 用0.02M的PBS清洗细胞4次，每次10min；

(13) DAB显色：取1ml三蒸水，加试剂盒中A、B、C试剂各一滴，混匀；室温显色，显微镜下控制反应时间，一般10min左右，及时用三蒸水中止反应；

(14)将用不同一抗的免疫组化结果在ScanScope XT (Aperio)观察和拍照。

(15)结果判定：根据染色强度及阳性细胞百分比对结果进行判定：阴性（－）：肿瘤细胞染色＜5%；弱阳性（+）：5%-10%；阳性（++）：10%-50%；强阳性（+++）：

＞50%。

## **2.3** 数据统计分析

数据采用EXCEL和SPSS16.0统计分析．*P*值*<*0.05认为统计差异显著。免疫组化中B-RAFV600E信号通路与Mps1相关性分析采用Spearman test。

# 第三章 实验结果

## **3.1** **Mps1**突变体对中心体复制及纺锤体形成的影响

前期研究发现B-RAFV600E能够通过磷酸化修饰Mps1增强其稳定性，导致过量Mps1蛋白在中心体处聚积。通过LC-MS\MS鉴定出Mps1上存在三个B-RAFV600E特异性磷酸化位点：S281、S436、S821。构建Mps1上B-RAFV600E相应磷酸化位点突变体，发现pS281-Mps1通过阻止Mps1与Cdc27的结合抑制由APC/CCdc20介导的Mps1蛋白泛素-蛋白酶体降解途径而增强其稳定性并导致过量Mps1蛋白聚积于中心体处。

为进一步明确Mps1上B-RAFV600E相应磷酸化位点对中心体复制及纺锤体形成的影响，利用pSUPER-Mps1逆转录病毒颗粒抑制293T细胞内源性Mps1蛋白的表达。以pEF36-HA-Mps1-WT及相应突变体（S281E、S436E、S821E、

EEE、AAA）转染293T细胞。Western bloting检测Mps1突变体蛋白的表达情况

（图1C）。48h后，以anti-γ和anti-αtublin抗体进行免疫荧光技术分析中心体数目异常及多极纺锤体形成（n≥3）细胞百分比。在293T细胞中过表达WT-Mps1不能引起中心体过度复制及多极纺锤体的形成，这与我们前期研究及其他在

293T细胞中的相关研究结果一致[37]。在过表达S821E、AAA的细胞中我们观察到了同样的结果。相反，在表达EEE、S281E、S436E细胞中发现多余中心体（n

≥3）及多极纺锤体形成。（图1A、B），表明pS281-Mps1、pS436-Mps1能够导致中心体过度复制及多极纺锤体的形成。更重要的是我们发现，表达EEE细胞对中心体复制及纺锤体形成的作用明显高于表达S281E、S436E 细胞，提示：

S281、S436两个位点同时磷酸化在肿瘤细胞有丝分裂异常中发挥了重要作用。

接着采用24h HU-arrest assay 将敲除了内源性Mps1 的293T 细胞和

SK-MEL31细胞阻断于有丝分裂S期，以pEF36-HA-Mps1-WT及相应突变体

（S281E、S436E、S821E、EEE、AAA）分别转染293T细胞和SK-MEL31细胞，

Western bloting检测Mps1突变体蛋白的表达情况（图1E）48h后，Leica DMI6000倒置荧光显微镜下观察中心体大小及数目，并计数中心体数目≥3细胞个数。因Mps1-WT 过表达也会引起中心体复制异常，我们拟以Mps1-AAA 突变体

（Mps1-AAA 不能被磷酸化而无法影响中心体复制）为阴性对照组。结果如图

1D所示，与表达WT-Mps1、S821E、AAA的细胞相比表达S281E、S436E、EEE

的细胞中心体复制异常（n≥3）细胞比例显著增高，依次为55%、30%、70%。综合以上结果表明Mps1模拟磷酸化位点S281和（或）S436能够导致中心

体复制异常及多极纺锤体的形成。



**Figure 1.** S281E is sufficient to trigger mitotic abnormalities. **A,** Endogenous Mps1 was depleted in 293T cells using pSUPER-siRNA as described before and then transfected with pEF36-HA-Mps1-W T or mutants. Fortyeight hours post transfection, cells were subjected to IF by using anti-γand anti-αtubulin antibodies. Bar graph shows the frequency of cells with three or more centrosomes. **B,** Bar graph shows the frequency of cells with multipolar spindles. **C,** Western blot shows the expression level of Mps1 constructs in 293T cells. **D,** Mps1-depleted 293T or SK-MEL31 cells were arrested in S-phase with a 24hr HU treatment, then transfected with Mps1-W T or mutants in the presence of HU. Centrosome number was determined 48h post transfection as described previously. E, Western blot shows the expression level of

Mps1 constructs in cells described in D. \* P＜0.05; \*\*P＜0.01.

## **3.2** **Mps1**突变体对**Mps1**蛋白激酶活性的影响

众所周知，磷酸化是蛋白质最重要的翻译后修饰之一，是包括Mps1在内许多蛋白激酶激活的主要机制[35-37]。因此，我们进一步分析了B-RAFV600E磷酸化修饰Mps1上相应三个特异性磷酸化位点：S281、S436、S821对Mps1激酶活性的影响。以MBP作为底物进行激酶活性检测，这里以kinase-dead（KD即不具有激酶活性）作为阴性对照，正如我们预期的一样，WT-Mps1有效磷酸化修饰MBP，而Mps1-KD则失活。S281E和S821E与野生型相比同样具有激酶活性。然而，S281A和S821A的Mps1激酶活性减弱甚微，S436A却显著抑制了Mps1

激酶活性，表明pS436-Mps1对Mps1激酶活性至关重要。S436E、EEE比WT-Mps1

具有更高的激酶活性（图2）。



**Figure 2 A,** 293T cells were transiently transfected with pHF36-GFP-Mps1 wild-type, KD, or mutants and treated with a proteasome inhibitor, MG115 (25µM), or the corresponding volume of DMSO. Twenty four hours post transfection, cell lysates were immunoprecipitated with anti-GFP antibody. Mps1 immunocomplexes were subjected to in vitro kinase assay following the previously described protocol (Mattison et al, 2007). Mps1 wild-type and KD were used as control. **B,** Mps1-associated in vitro kinase activity were quantified by ImageQuant 5.2 software.

## **3.3** **Mps1**突变体对染色体分离的影响

非整倍体是肿瘤细胞的典型特征，是人类肿瘤染色体不稳定性的普遍形式。人类细胞的正常染色体数量是46个或23对，而肿瘤细胞的染色体经常会少于

或者多于23对，即非整倍体细胞。

正常情况下，中心体仅在S期经历一次和染色体DNA复制类似的复制；在有丝分裂期，复制后的两个中心体分别指导两极纺锤体的形成，从而保证遗传物质在两个子细胞中的平均分配。因此，无论任何时候，细胞内仅含有一个（未复制前）或两个（复制后）中心体[40]。两个以上中心体的存在会增加有丝分裂错误的频率并使染色体分配不均衡，引起肿瘤细胞染色体不稳定性及非整倍体细胞出现。之前我们发现，Mps1突变体能够导致中心体复制异常及多极纺锤体的形成。而这些都是影响染色体稳定性的重要因素。我们推测Mps1突变体可以引起染色体不稳定性，非整倍体细胞的出现。

以pBabe-puro-HA-Mps1-EEE逆转录病毒颗粒感染Sbcl2细胞，SK-MEL31细胞，24h后，换条件培养基（含1µg/ml嘌呤霉素）继续培养7-10天，筛选阳性克隆，免疫荧光法验证Mps1突变体的表达，感染效率达90%以上，即为感染成功。96h后，进行metaphase-spreads analysis，结果表明，与空载体对照组相比，稳定表达Mps1-EEE的Sbcl2细胞，SK-MEL31细胞染色体分离异常细胞比例显著增高，其中，主要为多极纺锤体表型，其次亦有少数细胞出现一个或两个lagging-chromosomes.（图3A、B、C图4A、）。因此，我们有足够理由相信在稳定表达Mps1-EEE的Sbcl2细胞，SK-MEL31细胞中观察到的细胞有丝分裂异常导致了非整倍体细胞的产生。免疫荧光结果显示稳定表达Mps1-EEE的Sbcl2细胞，SK-MEL31细胞中染色体数目大于或小于46的细胞及非整倍体细胞比例显著增加。（图3D, 图4B）。综合以上结果得出如下结论，Mps1-EEE突变体能够导致染色体不稳定性，最终产生非整倍体细胞。



**Figure 3** Mps1-EEE drives mis-segregation of chromosomes and aneuploidy in human melanoma cells. Sbcl2 cells or primary human melanocytes were infected with pBabe-puro-HA-Mps1-EEE retrovirus. Sbcl2 cells infected with pBabe-puro-Mps1-EEE mutant or empty vector retrovirus were selected under puromycin (1mg/ml) for 10 days and subjected to metaphase spreads. The primary human melanocytes were infected with pBabe-puro-Mps1-EEE mutant or empty vector retrovirus. The efficiency was 90% as monitored by GFP fluorescence of a transfected GFP-containing plasmid. Ninety-six hours post infections, cells were subjected to metaphase spreads analysis. **A,** Bar graph shows the percentage of anaphase/telophase cells with normal versus abnormal chromosome segregation. Data was collected from 40-50anaphase/telophase cells per experiment and expressed as the mean±s. d. of three experiments. **B,** Examples of normal versus

Abnormalchromosome segregation in Sbcl2 (±Mps1-EEE). Cells were subjected to immunostaining with anti-a-tubulin to visualize microtubules (green). Chromosomes (blue) were detected by DAPI. Yellow arrows identify lagging chromosomes and chromosome bridges. **C,** Chromosome counts on metaphase spreads. Percent distribution of chromosome numbers obtained from at least 50 metaphase spreads. **D,** Representative photos of metaphase spreads from vector control and HA-Mps1-EEE-expressing Sbcl2 cells. Chromosomes were stainedwith DAPI and

Imaged aty 60 magnification.



**Figure 4**. Exogenous phospho-mimetic Mps1-EEE mutant expression drives mis-segregation of chromosomes and aneuploidy in SK-MEL31 cells. **A,** Examples of normal versus abnormal chromosome segregation in SK-MEL31 (+/-Mps1-EEE).

Cells were subjected to immunostaining with anti-α-tubulin to visualize microtubules (green). Chromosomes (blue) were detected by DAPI. Yellow arrows identify lagging chromosomes and chromosome bridges. **B,** Representative photos of metaphase spreads from vector control and HA-Mps1-EEE expressing SK-MEL31 cells. Chromosomes were stained with DAPI and imaged at×60 magnification.

## **3.4** **B-RAFV600E**/**MAPK**信号通路与**pS281-Mps1**在皮肤癌组织中的相关性分析

以上体外研究结果促使我们进一步探讨B-RAFV600E/MAPK信号通路与pS281-Mps1在人类皮肤癌组织中的相关性。从US Biomax, Inc (Rockville, MD)购买皮肤癌组织芯片(ME207)，该芯片包括正常皮肤对照及皮肤癌不同分期组织。以p-ERK（反映B-RAFV600E激酶活性）、p-S281 Mps1抗体做免疫组化分析，结果发现p-MAPK 阳性（包括阳性和强阳性）在正常皮肤组织（0%）、恶性黑色素瘤（48%）、转移黑色素瘤（69%）比例依次显著增高（P＜0.001）。同样pS281-Mps1阳性表达（包括阳性和强阳性）在不同组织中的百分比依次为：正常皮肤组织（0%）、恶性黑色素瘤（54%）、转移黑色素瘤（91%）（P＜0.001）

（图5）。并且结果显示p-MAPK阳性的病例同时显示pS281-Mps1阳性表达，反之亦然。提示二者在皮肤癌组织中显著相关，并与临床分期呈正相关（r＝0.25, P＝0.002, Spearman test）。



**Figure 5**. p-S281 Mps1 levels are correlated with p-MAPK in human melanoma tissues. (A, B) Immunohistochemistry (IHC) p-MAPK **A,** and p-S281 Mps1 protein **B,** staining of human malignant and metastatic melanomas on TMA (ME207). Original magnification 20×Representative examples of the whole-slide images, negative control, positive control, and human malignant melanoma with negative (A1), weak positive (A5), positive (C15), strong positive (D3) and human metastatic melanoma

With weak positive (I13), positive (G8), strong positive (G14). **C,** Column chart shows p-MAPK (left pane l) and p-S281 Mps1 (right pane l) immunoreactivity in human melanoma tissues using TMA (ME207).

# 第四章 讨 论

本研究受国家自然科学基金（30872932）资助，明确了B-RAFV600E引起肿瘤细胞中心体过度复制，产生非整倍体细胞，最终导致肿瘤发生的分子机制。B-RAFV600E通过磷酸化修饰S281-Mps1抑制由APC/CCdc20介导的Mps1蛋白泛素-蛋白酶体降解途径增强其蛋白稳定性。同时，我们发现B-RAFV600E通过磷酸化修饰S436-Mps1增强Mps1蛋白激酶活性，最终过量具有强激酶活性的Mps1蛋白聚积于中心体处，引起中心体过度复制、多极纺锤体形成、染色体分离异常。这些均是肿瘤细胞染色体不稳定性，非整倍体细胞产生的重要因素（图6）。以上结论在组织水平得到了验证，pS281-Mps1与p-MAPK在皮肤癌组织中显著相关，并与临床分期呈正相关（r＝0.25, P＝0.002, Spearman test）。本研究结果提示通过B-RAFV600E信号通路持续磷酸化Mps1在恶性肿瘤的发生发展中发挥了重要作用。



**Figure** **6** Schematic diagram of research **results.**

目前关于Mps1蛋白激酶是否参与中心体复制仍存在争议。但有研究发现有活性的Mps1蛋白存在于中心体处，过表达Mps1蛋白和抑制Mps1蛋白降解均能导致中心体过度复制[41]。我们研究发现在Mps1敲除的细胞中B-RAFV600E虽能引起纺锤体的分裂却不能引起中心体过度复制和多极纺锤体的形成。（**前期工作基础：图1**）提示：Mps1是BRAFV600E调控中心体过度复制及多极纺锤体形成的重要靶基因。

Mps1因其在中心体复制中发挥重要作用而得名，它也是纺锤体检测点复合物中的一员，其主要生物学功能是参与中心体的复制及纺锤体检测点[42]。纺锤

体检测点也在维持染色体稳定性中起重要作用，其功能缺陷将导致染色体单体错误分配，产生非整倍体子代细胞，最终导致肿瘤发生[43]。本研究采用HU-arrest

assay将细胞阻滞于有丝分裂S期，明确了B-RAFV600E信号通路通过调控Mps1蛋白稳定性及激酶活性导致纺锤体检测点功能及中心体复制异常。Mps1在有丝分裂M期具有最大激酶活性[32]。正常情况下，Mps1是一种极其不稳定的蛋白，中心体复制完毕及细胞进入Anaphase期后，在细胞内经由蛋白酶介导的泛素化途径降解[33]，纺锤体检测点功能关闭。在出芽酵母中，过表达Mps1能够在细胞有丝分裂后期重新激活Mps1通路下游分子功能及纺锤体检测点功能[44]，因此，在细胞有丝分裂后期B-RAFV600E抑制Mps1降解，引起在细胞有丝分裂后期/末期而不是细胞有丝分裂中期Mps1-dependent delay现象的发生。但有关B-RAFV600E导致细胞有丝分裂延迟的确切机制仍需进一步的探讨。

Mps1在细胞有丝分裂过程中高度磷酸化，而磷酸化是Mps1发挥功能的重要机制[45]。我们推测在细胞有丝分裂后期B-RAFV600E通过磷酸化修饰Mps1而增强其稳定性，本研究在293T细胞中证实了该假设（**前期工作基础：图2**）。有研究在爪蟾中发现MAPK信号通路对Mps1着丝粒定位能力有重要影响[46]，但与Mps1相关的上游或下游调控分子的鉴定未见报道。本研究发现B-RAFV600E是Mps1上游重要的调控分子。经LC-MS\MS鉴定出Mps1上存在三个B-RAFV600E特异性磷酸化位点：S281、S436、S821。癌基因B-RAFV600E通过磷酸化修饰单极纺锤体蛋白激酶Mps1-S281调控Mps1激酶的蛋白质稳定性。进一步机制研究发现我们研究结果发现S281E丧失Cdc27结合能力而不能将Mps1突变体呈递给APC/C进行泛素化降解。我们得出结论B-RAFV600E通过磷酸化修饰单极纺锤体蛋白激酶Mps1-S281阻止Mps1与Cdc27的相互作用增强其蛋白稳定性**（前期工作基础图3）。**

高浓度Mps1蛋白能够引起中心体过度复制[47]，目前已经发现在某些肿瘤细

胞中存在Mps1突变体，该突变体在细胞内无法经由泛素化途径降解，导致Mps1蛋白异常稳定，从而与肿瘤细胞中心体复制异常、纺锤体检测点功能异常密切相关[34]。在本研究中通过构建Mps1上B-RAFV600E相应磷酸化位点突变体，采用HU-arrest assay发现S281E和EEE能够导致中心体复制异常及多极纺锤体的形成。Daniel等[48]研究发现高浓度Mps1蛋白能够明显降低非整倍体肿瘤细胞的凋亡率，降低Mps1蛋白浓度导致肿瘤细胞凋亡，存活的细胞中非整倍体细胞比例明显降低。体内实验表明降低Mps1蛋白浓度能够抑制肿瘤的生长。我们研究结果显示B-RAFV600E磷酸化修饰Mps1增强其蛋白稳定性，我们推测B-RAFV600E可能通过调控Mps1蛋白稳定性在肿瘤细胞凋亡中发挥重要作用。

细胞分裂异常和染色体不稳定性是肿瘤细胞的典型特点[49]。中心体复制、纺锤体检测点是染色体稳定性的重要调控因素[50]。正常情况下，中心体仅在S期经历一次和染色体DNA复制类似的复制；在有丝分裂期，复制后的两个中心体分别指导两极纺锤体的形成，从而保证遗传物质在两个子细胞中的平均分配。因此，无论任何时候，细胞内仅含有一个（未复制前）或两个（复制后）中心体[51]。两个以上中心体的存在会增加有丝分裂错误的频率并使染色体分配不均衡，引起肿瘤细胞染色体不稳定性及非整倍体细胞出现[52]。我们研究结果显示稳定表达Mps1-EEE的Sbcl2细胞，SK-MEL31细胞染色体分离异常细胞比例显著增高，其中，主要为多极纺锤体表型，其次亦有少数细胞出现一个或两个落后染色体。因此，得出结论Mps1-EEE突变体能够导致染色体不稳定性，最终产生非整倍体细胞。

众所周知，磷酸化是蛋白质最重要的翻译后修饰之一，是包括Mps1在内许多蛋白激酶激活的主要机制[35-37]。因此，我们进一步分析了B-RAFV600E磷酸化修饰Mps1上相应三个特异性磷酸化位点：S281、S436、S821对Mps1激酶活性

的影响。以MBP作为底物进行激酶活性检测，S281E和S821E与野生型相比同样具有激酶活性。然而，S281A和S821A的Mps1激酶活性减弱甚微，S436A却显著抑制了Mps1激酶活性，表明S436E对Mps1激酶活性至关重要，且S436E、

EEE比WT-Mps1具有更高的激酶活性。

丝裂原活化蛋白激酶(MAPK)信号通路是生物体内重要的信号转导系统之一，参与介导细胞生长、发育、分裂和分化等多种生理及病理过程[53]。B-RAF基因属于RAF基因家族，编码一种丝/苏氨酸特异性激酶，是MAPK信号传导通路中RAS的下游作用底物、MEK的上游激酶，将信号从RAS转导至ERK。有研究报道RAS/RAF/ERK信号通路涉及了多个癌基因的激活，与肿瘤的发生发展密切相关。因此最后，我们进一步探讨B-RAFV600E信号通路与pS281-Mps1在人类肿瘤组织中的相关性。我们发现pS281-Mps1与p-MAPK在皮肤癌组织中显著相关，并与临床分期呈正相关（r＝0.25, P＝0.002, Spearman test）。可见，B-RAFV600E与Mps1在肿瘤发生发展中发挥了重要作用。

近年来肿瘤分子靶向治疗发展迅速，B-RAF基因在人类多种肿瘤组织中发生不同比例的突变，国外人群中在皮肤癌、甲状腺癌、结肠癌等恶性肿瘤中突变频率较高[41-43]。在中国人非肢端皮肤黑色素瘤中突变频率高达43.3%（13/30），且以V600E突变为主[44]。而该基因在中国人结直肠癌、甲状腺癌中的突变频率因样本量小而报道不一，分别约26%-65%、25-50%[45-46]。可见，B-RAF基因突变是目前人类多种恶性肿瘤中最为常见的基因变异，有可能成为靶向药物作用的靶点。

以结直肠癌为例，抗EGFR靶向治疗为结直肠癌患者带来了希望。多项临床研究表明，只有部分结直肠癌患者对抗EGFR靶向治疗有效如西昔妥单抗

（Cetuximab, Erbitux, C225, 爱必妥）竞争性与EGFR的胞外激酶特异性结合，并

且抑制EGFR与其它配体的结合，从而抑制下游信号通路的传导达到抗肿瘤的作用[54-56]。但在EGFR表达阳性的患者中C225的反应率仅能达到10%，最近的研究表明EGFR的表达、EGFR的突变状况并不能作为预测C225疗效的生物学标志

[57-58]。尽管K-RAS基因突变已经作为抗EGFR靶向治疗的一个预测指标，但并不

是所有K-RAS野生型患者都对该靶向治疗有效。现除KRAS外仍无一公认的生物学标志物来预测C225的疗效，所以仍需发现新的生物学标志来预测单抗的疗效。研究报道B-RAF基因与K-RAS基因虽然同处RAS-RAF-MAPK通路，但两者突变具有相互排它性，只有大约1%肿瘤同时存在RAS和B-RAF基因的突变[16]，因此，B-RAF基因能不依赖于K-RAS基因而独立参与肿瘤的发生发展过程，有可能成为靶向药物作用的靶点。多项研究表明K-RAS野生型而B-RAFV600E突变型患者对

EGFR单克隆抗体原发耐药，其无进展生存期和总生存率较B-RAF野生型都较短。B-RAF基因型与抗EGFR靶向治疗的疗效及预后密切相关，是抗EGFR靶向治疗的一个独立影响因素[18-19]。因此，迫切需要抗EGFR靶向治疗联合一些选择性B-RAF抑制剂（如PLX-4032和XL-281）的肿瘤治疗策略。

目前一些针对B-RAF激酶特异抑制剂已被成功应用于临床癌症治疗，例如BAY43-9006是RAF激酶特异性抑制剂，其在携带B-RAFV600E突变体黑色素瘤患者的临床治疗中显示显著疗效，但同时发现部分病人存在严重的BAY43-9006耐药性问题，甚至促进了肿瘤的发生发展[20-21]。进一步机制研究发现，尽管B-RAF特异性抑制剂可以抑制携带B-RAFV600E突变体细胞中MEK/ERK信号通路，但是该抑制剂反而可激活携带K-RAS基因突变及B-RAF野生型细胞中MEK/ERK通路。在这些细胞中由于B-RAF/C-RAF、C-RAF/C-RAF或B-RAF-KD/C-RAF等二聚体的形成，反而使MEK/ERK通路活性更强，从而促进肿瘤细胞增殖[22]。而且，RAF抑制剂不仅仅过度激活携带K-RAS基因突变细胞的MEK/ERK通路，对于那

些被其它癌基因（如HER2）激活的MEK/ERK通路，同样具有过度激活的作用

[23-24]. 另外，有报道COT基因[59]、IGF-1R/PI3K[60]途径等可能与RAF抑制剂耐药

性有关。尽管RAF激酶抑制剂在体外细胞水平的耐药机制研究2010年以来有所突破，但目前仍缺乏有效策略，尚需系统深入研究癌基因B-RAFV600E在肿瘤发生发展中的作用机制，并从中鉴定新的靶标基因，这对筛选新的药物靶点、指导临床联合使用药物策略有重要意义。

本研究发现癌基因B-RAFV600E通过磷酸化修饰有丝纺锤体监测点激酶Mps1-S281、S436、S821，分别影响Mps1激酶的蛋白质稳定性、激酶活性等，进而导致中心体过度复制、多极纺锤体出现，在肿瘤细胞染色体不稳定性、非整倍体细胞形成中发挥重要作用[30]，提示Mps1可能作为携带B-RAFV600E突变型恶性肿瘤新的治疗靶点。

更为重要的是本研究在美国人皮肤癌组织芯片中发现B-RAFV600E激酶活性与磷酸化修饰Mps1（p281-Mps1）蛋白水平显著相关，二者在皮肤癌组织中表达量较正常皮肤组织显著升高，与皮肤癌分期正相关。那么，二者在中国人皮肤癌、结直肠癌、甲状腺癌中是否相关？Mps1是否可作为新的预后预测指标？如上所述，目前迫切需要新的靶标基因作为个体化治疗的药物靶点，那么，Mps1基因是否可以作为携带B-RAFV600E突变型肿瘤患者新的治疗靶点？

未来，课题组将在本研究的基础上针对以上科学问题，大样本中国人皮肤癌、结直肠癌、甲状腺癌中分析B-RAFV600E与Mps1基因的相关性，并从细胞水平、动物水平观察基因敲除或Mps1激酶特异性抑制剂（Mps1-IN-1[61], NMS-P715[62]）对携带B-RAFV600E突变型恶性肿瘤新治疗的可能性，为携带B-RAFV600E突变型恶性肿瘤的临床治疗提供理论依据。（该部分工作已获得2012年国家自然科学基金青年基金（项目编号：81201956）的资助。



**B-RAFV600E**

**Stability**

**S281**

**S436**

**Mps1**

**Kinase activity**

**S821**

**Hyperactivity spindle checkpoint**

**Cell division failure**

**Supernumerary centrosomes**

**Multipolar spindles**

**Chromosome segregation error**

**We will investigatethe possibility of a therapeutic role of Mps1 inhibition**

**in patients with B-RAFV600E mutation through retrovirus infection,Mps1 inhibitor, and**

**Mps1 siRNA.**

**Aneuploidy & chromosome instability**

**Study has been completed**

**Future research (NSFY:81201956)**

**Figure** **7** Academic schematic diagram

# 第五章 结 论

癌基因B-RAFV600E通过磷酸化修饰有丝纺锤体监测点激酶Mps1-S281、

S436、S821，分别影响Mps1激酶的蛋白质稳定性、激酶活性等，进而导致中心体过度复制、多极纺锤体出现，在肿瘤细胞染色体不稳定性、非整倍体细胞形成中发挥重要作用

参考文献：

[1]. Wan PT, Garnett MJ, Roe SM, Lee S, Niculescu-Duvaz D, Good VM, Jones CM, Marshall CJ, Springer CJ, Barford D, Marais R. Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-Raf. Cell. 2004.116(6), 855-867.

[2]. Dumaz N. Mechanism of RAF isoform switching induced by oncogenic RAS in melanoma. Small Gtpases. 2011. 2(5), 289-292.

3. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, Davis N, Dicks E, Ewing R, Floyd Y, Gray K, Hall S, Hawes R, Hughes J, Kosmidou V, Menzies A, Mould C, Parker A, Stevens C, Watt S, Hooper S, Wilson R, Jayatilake H, Gusterson BA, Cooper C, Shipley J, Hargrave D, Pritchard–Jones K, Maitland N, Chenevix-Trench G, Rigins GL, Bigner DD, Palmieri G, Cossu A, Flanagan A, Nicholson A, Ho JW, Leung SY, Yuen ST, Weber BL, Seigler HF, Darrow TL, Paterson H, Marais R, Marshall CJ, Wooster R, Stratton MR, Futreal PA. Mutations of the BRAF gene in human cancer. Nature. 2002.417(6892), 949–954.

[4. Anderson S](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Anderson%20S%22%5BAuthor%5D), [Bloom KJ](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Bloom%20KJ%22%5BAuthor%5D), [Vallera DU](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Vallera%20DU%22%5BAuthor%5D), [Rueschoff J](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Rueschoff%20J%22%5BAuthor%5D), [Meldrum C](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Meldrum%20C%22%5BAuthor%5D), [Schilling R](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Schilling%20R%22%5BAuthor%5D), [Kovach B](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Kovach%20B%22%5BAuthor%5D), [Lee JR](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Lee%20JR%22%5BAuthor%5D), [Ochoa P](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Ochoa%20P%22%5BAuthor%5D), [Langland R](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Langland%20R%22%5BAuthor%5D), [Halait H](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Halait%20H%22%5BAuthor%5D), [Lawrence HJ](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Lawrence%20HJ%22%5BAuthor%5D), [Dugan MC](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Dugan%20MC%22%5BAuthor%5D). Multisite Analytic Performance Studies of a Real-Time Polymerase Chain Reaction Assay for the Detection of BRAF V600E Mutations in Formalin-Fixed Paraffin-Embedded Tissue Specimens of Malignant Melanoma. [Arch Pathol Lab Med.](http://www.ncbi.nlm.nih.gov/pubmed/22332713#%23) 2012. [Epub ahead of print]

[5. Kim SJ](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Kim%20SJ%22%5BAuthor%5D), [Lee KE](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Lee%20KE%22%5BAuthor%5D), [Myong JP](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Myong%20JP%22%5BAuthor%5D), [Park JH](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Park%20JH%22%5BAuthor%5D), [Jeon YK](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Jeon%20YK%22%5BAuthor%5D), [Min HS](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Min%20HS%22%5BAuthor%5D), [Park SY](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Park%20SY%22%5BAuthor%5D), [Jung KC](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Jung%20KC%22%5BAuthor%5D), [Koo do H](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Koo%20do%20H%22%5BAuthor%5D), [Youn YK](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Youn%20YK%22%5BAuthor%5D). BRAF(V600E) Mutation is Associated with Tumor Aggressiveness in Papillary Thyroid Cancer. [World J Surg.](http://www.ncbi.nlm.nih.gov/pubmed/22190222#%23) 2012. 36(2):310-7.

[6. Kalady MF](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Kalady%20MF%22%5BAuthor%5D), [Dejulius KL](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Dejulius%20KL%22%5BAuthor%5D), [Sanchez JA](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Sanchez%20JA%22%5BAuthor%5D), [Jarrar A](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Jarrar%20A%22%5BAuthor%5D), [Liu X](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Liu%20X%22%5BAuthor%5D), [Manilich E](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Manilich%20E%22%5BAuthor%5D), [Skacel M](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Skacel%20M%22%5BAuthor%5D), [Church JM](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Church%20JM%22%5BAuthor%5D). BRAF mutations in colorectal cancer are associated with distinct clinical characteristics and worse prognosis. [Dis Colon Rectum.](http://www.ncbi.nlm.nih.gov/pubmed/22228154) 2012. 55(2), 128-33.

[7. Wong KK](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Wong%20KK%22%5BAuthor%5D), [Tsang YT](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Tsang%20YT%22%5BAuthor%5D), [Deavers MT](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Deavers%20MT%22%5BAuthor%5D), [Mok SC](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Mok%20SC%22%5BAuthor%5D), [Zu Z](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Zu%20Z%22%5BAuthor%5D), [Sun C](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Sun%20C%22%5BAuthor%5D), [Malpica A](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Malpica%20A%22%5BAuthor%5D), [Wolf JK](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Wolf%20JK%22%5BAuthor%5D), [Lu KH](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Lu%20KH%22%5BAuthor%5D), [Gershenson DM](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Gershenson%20DM%22%5BAuthor%5D). BRAF mutation is rare in advanced-stage low-grade ovarian serous carcinomas. [Am J Pathol.](http://www.ncbi.nlm.nih.gov/pubmed/20802181#%23) 2010 177(4), 1611-7.

[8. Colombino M](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Colombino%20M%22%5BAuthor%5D), [Sperlongano P](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Sperlongano%20P%22%5BAuthor%5D), [Izzo F](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Izzo%20F%22%5BAuthor%5D), [Tatangelo F](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Tatangelo%20F%22%5BAuthor%5D), [Botti G](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Botti%20G%22%5BAuthor%5D), [Lombardi A](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Lombardi%20A%22%5BAuthor%5D), [Accardo M](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Accardo%20M%22%5BAuthor%5D), [Tarantino L](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Tarantino%20L%22%5BAuthor%5D), [Sordelli I](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Sordelli%20I%22%5BAuthor%5D), [Agresti M](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Agresti%20M%22%5BAuthor%5D), [Abbruzzese A](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Abbruzzese%20A%22%5BAuthor%5D), [Caraglia M](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Caraglia%20M%22%5BAuthor%5D), [Palmieri G](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Palmieri%20G%22%5BAuthor%5D). BRAF and PIK3CA genes are somatically mutated in hepatocellular carcinoma among patients from South Italy. [Cell Death Dis.](http://www.ncbi.nlm.nih.gov/pubmed/22258409#%23) 2012. 3: e259

[9. Kobayashi M](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Kobayashi%20M%22%5BAuthor%5D), [Sonobe M](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Sonobe%20M%22%5BAuthor%5D), [Takahashi T](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Takahashi%20T%22%5BAuthor%5D), [Yoshizawa A](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Yoshizawa%20A%22%5BAuthor%5D), [Ishikawa M](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Ishikawa%20M%22%5BAuthor%5D), [Kikuchi R](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Kikuchi%20R%22%5BAuthor%5D), [Okubo K](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Okubo%20K%22%5BAuthor%5D), [Huang CL](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Huang%20CL%22%5BAuthor%5D), [Date H](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Date%20H%22%5BAuthor%5D). Clinical significance of BRAF gene mutations in patients with non-small cell lung cancer. [Anticancer Res.](http://www.ncbi.nlm.nih.gov/pubmed/22199339#%23) 2011. 31(12), 4619-23.

[10. Greenman C](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Greenman%20C%22%5BAuthor%5D), [Stephens P](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Stephens%20P%22%5BAuthor%5D), [Smith R](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Smith%20R%22%5BAuthor%5D), [Dalgliesh GL](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Dalgliesh%20GL%22%5BAuthor%5D), [Hunter C](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Hunter%20C%22%5BAuthor%5D), [Bignell G](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Bignell%20G%22%5BAuthor%5D), [Davies H](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Davies%20H%22%5BAuthor%5D), [Teague J](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Teague%20J%22%5BAuthor%5D), [Butler A](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Butler%20A%22%5BAuthor%5D), [Stevens C](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Stevens%20C%22%5BAuthor%5D), [Edkins S](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Edkins%20S%22%5BAuthor%5D), [O'Meara S](http://www.ncbi.nlm.nih.gov/pubmed?term=%22O%27Meara%20S%22%5BAuthor%5D), [Vastrik I](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Vastrik%20I%22%5BAuthor%5D), [Schmidt EE](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Schmidt%20EE%22%5BAuthor%5D), [Avis T](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Avis%20T%22%5BAuthor%5D), [Barthorpe S](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Barthorpe%20S%22%5BAuthor%5D), [Bhamra G](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Bhamra%20G%22%5BAuthor%5D), [Buck G](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Buck%20G%22%5BAuthor%5D), [Choudhury B](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Choudhury%20B%22%5BAuthor%5D), [Clements J](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Clements%20J%22%5BAuthor%5D), [Cole J](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Cole%20J%22%5BAuthor%5D), [Dicks E](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Dicks%20E%22%5BAuthor%5D), [Forbes S](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Forbes%20S%22%5BAuthor%5D), [Gray K](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Gray%20K%22%5BAuthor%5D), [Halliday K](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Halliday%20K%22%5BAuthor%5D), [Harrison R](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Harrison%20R%22%5BAuthor%5D), [Hills K](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Hills%20K%22%5BAuthor%5D), [Hinton J](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Hinton%20J%22%5BAuthor%5D), [Jenkinson A](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Jenkinson%20A%22%5BAuthor%5D), [Jones D](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Jones%20D%22%5BAuthor%5D), [Menzies A](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Menzies%20A%22%5BAuthor%5D), [Mironenko T](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Mironenko%20T%22%5BAuthor%5D), [Perry J](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Perry%20J%22%5BAuthor%5D), [Raine K](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Raine%20K%22%5BAuthor%5D), [Richardson D](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Richardson%20D%22%5BAuthor%5D), [Shepherd R](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Shepherd%20R%22%5BAuthor%5D), [Small A](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Small%20A%22%5BAuthor%5D), [Tofts C](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Tofts%20C%22%5BAuthor%5D), [Varian](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Varian%20J%22%5BAuthor%5D) J, [Webb T](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Webb%20T%22%5BAuthor%5D), [West S](http://www.ncbi.nlm.nih.gov/pubmed?term=%22West%20S%22%5BAuthor%5D), [Widaa S](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Widaa%20S%22%5BAuthor%5D), [Yates A](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Yates%20A%22%5BAuthor%5D), [Cahill DP](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Cahill%20DP%22%5BAuthor%5D), [Louis DN](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Louis%20DN%22%5BAuthor%5D), [Goldstraw P](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Goldstraw%20P%22%5BAuthor%5D), [Nicholson AG](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Nicholson%20AG%22%5BAuthor%5D), [Brasseur F](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Brasseur%20F%22%5BAuthor%5D), [Looijenga L](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Looijenga%20L%22%5BAuthor%5D), [Weber BL](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Weber%20BL%22%5BAuthor%5D), [Chiew YE](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Chiew%20YE%22%5BAuthor%5D), [DeFazio A](http://www.ncbi.nlm.nih.gov/pubmed?term=%22DeFazio%20A%22%5BAuthor%5D), [Greaves MF](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Greaves%20MF%22%5BAuthor%5D), [Green AR](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Green%20AR%22%5BAuthor%5D), [Campbell P](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Campbell%20P%22%5BAuthor%5D), [Birney E](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Birney%20E%22%5BAuthor%5D), [Easton DF](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Easton%20DF%22%5BAuthor%5D), [Chenevix-Trench G](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Chenevix-Trench%20G%22%5BAuthor%5D), [Tan MH](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Tan%20MH%22%5BAuthor%5D), [Khoo SK](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Khoo%20SK%22%5BAuthor%5D), [Teh BT](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Teh%20BT%22%5BAuthor%5D), [Yuen ST](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Yuen%20ST%22%5BAuthor%5D), [Leung SY](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Leung%20SY%22%5BAuthor%5D), [Wooster R](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Wooster%20R%22%5BAuthor%5D), [Futreal PA](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Futreal%20PA%22%5BAuthor%5D), [Stratton MR](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Stratton%20MR%22%5BAuthor%5D). Patterns of somatic mutation in human cancer genomes. [Nature.](http://www.ncbi.nlm.nih.gov/pubmed/17344846#%23) 2007 446(7132), 153-8.

[11. Si L](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Si%20L%22%5BAuthor%5D), [Kong Y](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Kong%20Y%22%5BAuthor%5D), [Xu X](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Xu%20X%22%5BAuthor%5D), [Flaherty KT](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Flaherty%20KT%22%5BAuthor%5D), [Sheng X](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Sheng%20X%22%5BAuthor%5D), [Cui C](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Cui%20C%22%5BAuthor%5D), [Chi Z](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Chi%20Z%22%5BAuthor%5D), [Li S](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Li%20S%22%5BAuthor%5D), [Mao L](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Mao%20L%22%5BAuthor%5D), [Guo J](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Guo%20J%22%5BAuthor%5D). Prevalence of BRAF V600E mutation in Chinese melanoma patients: large scale

Analysis of BRAF and NRAS mutations in a 432-case cohort. [Eur J Cancer.](http://www.ncbi.nlm.nih.gov/pubmed/21788131#%23) 2012. 48(1), 94-100.

12. Liou JM, Wu MS, Shun CT, Chiu HM, Chen MJ, Chen CC, Wang HP, Lin JT, Liang JT. [Mutations in BRAF correlate with poor survival of colorectal cancers in Chinese population.](http://www.ncbi.nlm.nih.gov/pubmed/21553007) Int J Colorectal Dis. 2011. 26(11):1387-95.

[13. Dhomen N](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Dhomen%20N%22%5BAuthor%5D), [Reis-Filho JS](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Reis-Filho%20JS%22%5BAuthor%5D), [da Rocha Dias S](http://www.ncbi.nlm.nih.gov/pubmed?term=%22da%20Rocha%20Dias%20S%22%5BAuthor%5D), et al. Oncogenic Braf induces melanocyte senescence and melanoma in mice. Cancer Cell, 2009, 15: 294-303.

[14. Saridaki Z](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Saridaki%20Z%22%5BAuthor%5D), [Papadatos-Pastos D](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Papadatos-Pastos%20D%22%5BAuthor%5D), [Tzardi M](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Tzardi%20M%22%5BAuthor%5D), [Mavroudis D](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Mavroudis%20D%22%5BAuthor%5D), [Bairaktari E](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Bairaktari%20E%22%5BAuthor%5D), [Arvanity H](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Arvanity%20H%22%5BAuthor%5D), [Stathopoulos E](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Stathopoulos%20E%22%5BAuthor%5D), [Georgoulias V](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Georgoulias%20V%22%5BAuthor%5D), [Souglakos J](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Souglakos%20J%22%5BAuthor%5D). BRAF mutations, microsatellite instability status and cyclin D1 expression predict metastatic colorectal patients' outcome. [Br J Cancer.](http://www.ncbi.nlm.nih.gov/pubmed/20485284#%23) 2010. 102(12), 1762-8.

[15. Perera PM](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Perera%20PM%22%5BAuthor%5D), [Wypasek E](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Wypasek%20E%22%5BAuthor%5D), [Madhavan S](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Madhavan%20S%22%5BAuthor%5D), [Rath-Deschner B](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Rath-Deschner%20B%22%5BAuthor%5D), [Liu J](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Liu%20J%22%5BAuthor%5D), [Nam J](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Nam%20J%22%5BAuthor%5D), [Rath B](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Rath%20B%22%5BAuthor%5D), [Huang Y](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Huang%20Y%22%5BAuthor%5D), [Deschner J](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Deschner%20J%22%5BAuthor%5D), [Piesco N](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Piesco%20N%22%5BAuthor%5D), [Wu C](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Wu%20C%22%5BAuthor%5D), [Agarwal S](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Agarwal%20S%22%5BAuthor%5D). Mechanical signals control SOX-9, VEGF, and c-Myc expression and cell proliferation during inflammation via integrin-linked kinase, B-Raf, and ERK1/2-dependent signaling in articular chondrocytes. [Arthritis Res Ther.](http://www.ncbi.nlm.nih.gov/pubmed/20509944#%23) 2010. 12(3):R106.

[Jinushi M](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Jinushi%20M%22%5BAuthor%5D), [Chiba S](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Chiba%20S%22%5BAuthor%5D), [Baghdadi M](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Baghdadi%20M%22%5BAuthor%5D), [Kinoshita I](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Kinoshita%20I%22%5BAuthor%5D), [Dosaka-Akita H](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Dosaka-Akita%20H%22%5BAuthor%5D), [Ito K](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Ito%20K%22%5BAuthor%5D), [Yoshiyama H](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Yoshiyama%20H%22%5BAuthor%5D), [Yagita H](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Yagita%20H%22%5BAuthor%5D), [Uede T](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Uede%20T%22%5BAuthor%5D), [Takaoka A](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Takaoka%20A%22%5BAuthor%5D). ATM-mediated DNA damage signals mediate immune escape through integrin-αvβ3-dependent mechanisms. [Cancer Res.](http://www.ncbi.nlm.nih.gov/pubmed?term=ATM-Mediated%20DNA%20Damage%20Signals%20Mediate%20Immune%20Escape%20through%20Integrin-%CE%B1v%CE%B23%E2%80%93Dependent%20Mechanisms) 2012. 72(1), 56-65.

[16. Naguib A](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Naguib%20A%22%5BAuthor%5D), [Mitrou PN](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Mitrou%20PN%22%5BAuthor%5D), [Gay LJ](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Gay%20LJ%22%5BAuthor%5D), [Cooke JC](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Cooke%20JC%22%5BAuthor%5D), [Luben RN](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Luben%20RN%22%5BAuthor%5D), [Ball RY](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Ball%20RY%22%5BAuthor%5D), [McTaggart A](http://www.ncbi.nlm.nih.gov/pubmed?term=%22McTaggart%20A%22%5BAuthor%5D), [Arends MJ](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Arends%20MJ%22%5BAuthor%5D), [Rodwell SA](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Rodwell%20SA%22%5BAuthor%5D). Dietary, lifestyle and clinicopathological factors associated with BRAF and K-ras mutations arising in distinct subsets of colorectal cancers in the EPIC Norfolk study. [BMC Cancer.](http://www.ncbi.nlm.nih.gov/pubmed/20233436#%23) 2010. 16;10:99.

17. Ikenoue T, Hikiba Y, Kanai F, et al. Diffrent effects of point mutations within the B-Raf glycine-rich loopin colorectal tumors on mitogen-activated protein/extracellular signal-regulated kinase kinase/extracellular signal-regulat ed kinase and nuclear factorκB pathway and cellular transformation. Cancer Res. 2004,64:3428-3435

18. M. K. Borysova, Y. Cui, M. Snyder, and T. M. Guadagno\*. Knockdown of B-Raf impairs spindle formation and the mitotic checkpoint in human somatic cells. Cell Cycle. 2008.7(18), 2894-2901.

19. Cui Y, Borysova MK, Johnson JO, Guadagno TM. Oncogenic B-RafV600EInduces Spindle Abnormalities, Supernumerary Centrosomes, an Aneuploidy in Human Melanocytic Cells. Cancer Research. 2010.70(2), 675-684.

20. Jing Liu, Xiaolong Cheng, Yanyan Zhang, Shujing Li, Heyang Cui, Ling Zhang, Ruyi Shi, Zhiping Zhao, Chanting He, Chuangui Wang, Haoliang Zhao, Ce Zhang, Harold A. Fisk, Thomas M. Guadagno, Yongping Cui\*. Phosphorylation of Mps1 by BRAFV600E Prevents Mps1 Degradation and Contributes to Chromosome Instability in Melanoma. Oncogene. 2013. 7;32(6):713-23.

[21. Tímár J](http://www.ncbi.nlm.nih.gov/pubmed?term=%22T%C3%ADm%C3%A1r%20J%22%5BAuthor%5D), [Hegedüs B](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Heged%C3%BCs%20B%22%5BAuthor%5D), [RásóE](http://www.ncbi.nlm.nih.gov/pubmed?term=%22R%C3%A1s%C3%B3%20E%22%5BAuthor%5D). KRAS mutation testing of colorectal cancer for anti-EGFR therapy: dogmas versus evidence. [Curr Cancer Drug Targets.](http://www.ncbi.nlm.nih.gov/pubmed/20718705#%23) 2010.10(8), 813-23.

[22. Prahallad A](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Prahallad%20A%22%5BAuthor%5D), [Sun C](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Sun%20C%22%5BAuthor%5D), [Huang S](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Huang%20S%22%5BAuthor%5D), [Di Nicolantonio F](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Di%20Nicolantonio%20F%22%5BAuthor%5D), [Salazar R](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Salazar%20R%22%5BAuthor%5D), [Zecchin D](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Zecchin%20D%22%5BAuthor%5D), [Beijersbergen RL](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Beijersbergen%20RL%22%5BAuthor%5D), [Bardelli A](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Bardelli%20A%22%5BAuthor%5D), [Bernards R](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Bernards%20R%22%5BAuthor%5D). Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. [Nature.](http://www.ncbi.nlm.nih.gov/pubmed/22281684#%23) 2012. 483(7387), 100-3.

[23. Königsberg R](http://www.ncbi.nlm.nih.gov/pubmed?term=%22K%C3%B6nigsberg%20R%22%5BAuthor%5D), [Hulla W](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Hulla%20W%22%5BAuthor%5D), [Klimpfinger M](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Klimpfinger%20M%22%5BAuthor%5D), [Reiner-Concin A](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Reiner-Concin%20A%22%5BAuthor%5D), [Steininger T](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Steininger%20T%22%5BAuthor%5D), [Büchler W](http://www.ncbi.nlm.nih.gov/pubmed?term=%22B%C3%BCchler%20W%22%5BAuthor%5D), [Terkola R](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Terkola%20R%22%5BAuthor%5D), [Dittrich C](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Dittrich%20C%22%5BAuthor%5D). Clinical and Economic Aspects of KRAS Mutational Status as Predictor for Epidermal Growth Factor Receptor Inhibitor Therapy in Metastatic Colorectal Cancer Patients. [Oncology.](http://www.ncbi.nlm.nih.gov/pubmed/22248908#%23) 2011. 81(5-6):359-64.

24. Weeraratna AT. [RAF around the edges--the paradox of BRAF inhibitors.](http://www.ncbi.nlm.nih.gov/pubmed/22256810) N Engl J Med. 2012.366(3):271-3.

25. Su F, Viros A, Milagre C, Trunzer K, Bollag G, Spleiss O, Reis-Filho JS, Kong X, Koya RC, Flaherty KT, Chapman PB, Kim MJ, Hayward R, Martin M, Yang H, Wang Q, Hilton H, Hang JS, Noe J, Lambros M, Geyer F, Dhomen N, Niculescu-Duvaz I, Zambon A, Niculescu-Duvaz D, Preece N, Robert L, Otte NJ, Mok S, Kee D, Ma Y, Zhang C, Habets G, Burton EA, Wong B, Nguyen H, Kockx M, Andries L, Lestini B, Nolop KB, Lee RJ, Joe AK, Troy JL, Gonzalez R, Hutson TE, Puzanov I, Chmielowski B, Springer CJ, McArthur GA, Sosman JA, Lo RS, Ribas A, Marais R. [RAS mutations in cutaneous squamous-cell carcinomas in patients treated with BRAF inhibitors.](http://www.ncbi.nlm.nih.gov/pubmed/22256804) N Engl J Med. 2012.366(3):207-15.

26. Heath EM, Kaufman KL, Christopherson RI. B-RAF: A contributor to the melanoma phenotype. Int J Biochem Cell Biol. 2011. 43(1), 29-32.

27. Wajapeyee N, Serra RW, Zhu X, Mahalingam M., Green MR. Role for IGFBP7 in senescence induction by BRAF. Cell. 2010. 141(5),746-7.

28. Bommarito A, Richiusa P, Carissimi E, Pizzolanti G, Rodolico V, Zito G, Criscimanna A, Di Blasi F, Pitrone M, Zerilli M, Amato MC, Spinelli G, Carina V, Modica G, Latteri MA, Galluzzo A, Giordano C. BRAFV600E mutation, TIMP-1 upregulation, and NF-κB activation: closing the loop on the papillary thyroid cancer trilogy. Endocr Relat Cancer. 2011, 18(6), 669-85.

29. Patton EE, Widlund HR, Kutok JL, Kopani KR, Amatruda JF, Murphey RD, Berghmans S, Mayhall EA, Traver D, Fletcher CD, Aster JC, Granter SR, Look AT, Lee C, Fisher DE, Zon LI. B-RAF mutations are sufficient to promote nevi formation and cooperate with p53 in the genesis of melanoma. Curr Biol. 2005.15(3), 249-254.

30. Lee SJ, Lee MH, Kim DW, Lee S, Huang S, Ryu MJ, Kim YK, Kim SJ, Kim SJ, Hwang JH, Oh S, Cho H, Kim JM, Lim DS, Jo YS, Shong M. Cross-regulation between oncogenic BRAF(V600E) kinase and the MST1 pathway in papillary thyroid carcinoma. PLoS One. 2011.6(1), e16180.

31. Cui Y, Guadagno TM. B-RafV600E signaling deregulates the mitotic spindle checkpoint throughstabilizing Mps1 levels in melanoma cells. Oncogene. 2008.27(22), 3122-3133.

32. Malumbres M, Barbacid M. Cell cycle kinases in cancer. Curr opin Genet Dev. 2007, 17: 60 -65.

33. Palframan WJ, Meehi JB, Jaspersen SL, Winey M, Murray AW. Anaphase inactivation of the spindle checkpoint. Science. 2006, 313: 680-684.

34. Kasbek C., Yang CH., Yusof AM., Chapman HM., Winey M., and Fisk HA. Preventing the degradation of Mps1 at centrosomes is sufficient to cause centrosome reduplication in human cells. Mol Biol Cell. 2007, 18: 4457-4469 .

35. Tyler RK, Chu ML, Johnson H, McKenzie EA, Gaskell SJ, Eyers PA. Phosphoregulation of human Mps1 kinase. Biochem J. 2009, 417:173-81.

36. Mattison CP, Old WM, Steiner E, Huneycutt BJ, Resing KA, Ahn NG et al. Mps1 activation loop autophosphorylation enhances kinase activity. J Biol Chem 2007, 282: 30553-30561.

37. Kang J, Chen Y, Zhao Y, Yu H. Autophosphorylation-dependent activation of

Human Mps1 is required for the spindle checkpoint. Proc Natl Acad Sci USA. 2007,104: 20232-20237.

38. Jefford CE, Irminger—Finger I. Mechanisms of chromosome instability in cancers. Crit Rev Oncol Hematol , 2006, 59: 1-14.

39. Tsou MF, Stearns T. Mechanism limiting centrosome duplication to once per cell cycle. Nature, 2006, 442: 947-951.

40. Srsen V, Merds A. The centrosome and cell proliferation. Cell Div, 2006, 1: 2 6.

41. Fisk HA, Mattison CP, Winey M. Human Mps1 protein kinase is required for centrosome duplication and normal mitotic progressi on. PNAS. 2003, 100: 14875-80.

42. Fisk HA, Winey M. Spindle regulation: Mps1 files into new areas. Curr Biol. 2004, 14: R1058-60.

43. Hoyt MA. Cell biology. Extinguishing a cell cycle checkpoint. Science. 2006, 313: 624-625.

44. Palframan WJ, Meehi JB, Jaspersen SL, Winey M, Murray AW. Anaphase inactivation of the spindle checkpoint. Science. 2006, 313:680-684.

45. Jelluma N, Brenkman AB, van den Broek NJ, Cruijsen CW, van Osch MH, Lens SM et al. Mps1 phosphorylates Borealin to control Aurora B activity and chromosome alignment. Cell. 2008, 132:233-246.

46. Zhao Y, Chen RH. Mps1 phosphorylation by MAP kinase is required for kinetochore localization of spindle-checkpoint proteins. Curr Biol. 2006, 16:1764-1769.

47. Pike AN, Fisk HA. Centriole assembly and the role of Mps1: defensible or

DispensableCellDiv. 2011, 6:9.

48. Daniel J, Coulter J, Woo JH,. High levels of the Mps1 checkpoint protein are protective of aneuploidy in breast cancer cells. Proc Natl Acad Sci U S A. 2011.108(13):5384-5389

49. Jefford CE, Irminger—Finger I. Mechanisms of chromosome instability in cancers. Crit Rev Oncol Hematol , 2006, 59: 1-14.

50. Tsou MF, Stearns T. Mechanism limiting centrosome duplication to once per cell cycle. Nature, 2006, 442: 947-951.

51. Srsen V, Merds A. The centrosome and cell proliferation. Cell Div, 2006, 1: 2 6.

52. Bettencourt-Di as M, Glover DM. Centrosome biogenesis and function: centrosomics brings new understanding. Nat Rv Mol Cell Bio, 2007, 8: 451-463.

[53. Zhou Y](http://www.ncbi.nlm.nih.gov/pubmed?term=Zhou%20Y%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23501044), [Zhang M](http://www.ncbi.nlm.nih.gov/pubmed?term=Zhang%20M%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23501044), [Sun GY](http://www.ncbi.nlm.nih.gov/pubmed?term=Sun%20GY%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23501044), [Liu YP](http://www.ncbi.nlm.nih.gov/pubmed?term=Liu%20YP%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23501044), [Ran WZ](http://www.ncbi.nlm.nih.gov/pubmed?term=Ran%20WZ%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23501044), [Peng L](http://www.ncbi.nlm.nih.gov/pubmed?term=Peng%20L%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23501044), [Guan CX](http://www.ncbi.nlm.nih.gov/pubmed?term=Guan%20CX%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23501044). Calcitonin gene-related peptide promotes the wound healing of human bronchial epithelial cells via PKC andMAPK pathways. [Regul Pept.](http://www.ncbi.nlm.nih.gov/pubmed/23501044) 2013 Mar 14. pii: S0167-0115(13) 00049-9.

[54. Woo J](http://www.ncbi.nlm.nih.gov/pubmed?term=Woo%20J%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23504768), [Palmisiano N](http://www.ncbi.nlm.nih.gov/pubmed?term=Palmisiano%20N%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23504768), [Tester W](http://www.ncbi.nlm.nih.gov/pubmed?term=Tester%20W%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23504768), [Leighton JC Jr](http://www.ncbi.nlm.nih.gov/pubmed?term=Leighton%20JC%20Jr%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23504768). Controversies in antiepidermal growth factor receptor therapy in metastatic colorectal cancer. [Cancer.](http://www.ncbi.nlm.nih.gov/pubmed/23504768) 2013 Mar 15. doi: 10.1002

[55. Xynos ID](http://www.ncbi.nlm.nih.gov/pubmed?term=Xynos%20ID%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23445638), [Karadima ML](http://www.ncbi.nlm.nih.gov/pubmed?term=Karadima%20ML%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23445638), [Voutsas IF](http://www.ncbi.nlm.nih.gov/pubmed?term=Voutsas%20IF%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23445638), [Amptoulach S](http://www.ncbi.nlm.nih.gov/pubmed?term=Amptoulach%20S%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23445638), [Skopelitis E](http://www.ncbi.nlm.nih.gov/pubmed?term=Skopelitis%20E%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23445638), [Kosmas C](http://www.ncbi.nlm.nih.gov/pubmed?term=Kosmas%20C%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23445638), [Gritzapis AD](http://www.ncbi.nlm.nih.gov/pubmed?term=Gritzapis%20AD%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23445638), [Tsavaris N](http://www.ncbi.nlm.nih.gov/pubmed?term=Tsavaris%20N%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23445638). Chemotherapy±Cetuximab Modulates Peripheral Immune Responses in Metastatic Colorectal Cancer. [Oncology.](http://www.ncbi.nlm.nih.gov/pubmed/23445638) 2013 Feb 22;84(5):273-283.

[56. Jiang Z](http://www.ncbi.nlm.nih.gov/pubmed?term=Jiang%20Z%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23441167), [Li C](http://www.ncbi.nlm.nih.gov/pubmed?term=Li%20C%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23441167), [Li F](http://www.ncbi.nlm.nih.gov/pubmed?term=Li%20F%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23441167), [Wang X](http://www.ncbi.nlm.nih.gov/pubmed?term=Wang%20X%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23441167)EGFR Gene Copy Number as a Prognostic Marker

In Colorectal Cancer Patients Treated with Cetuximab or Panitumumab: A Systematic Review and Meta Analysis. [PLoS One.](http://www.ncbi.nlm.nih.gov/pubmed/23441167) 2013;8(2):e56205. doi: 10.1371

57. Tebbutt NC, Parry MM, Zannino D, Strickland AH, Van Hazel GA, Pavlakis N, Ganju V, Mellor D, Dobrovic A, Gebski VJ. Docetaxel plus cetuximab as second-line treatment for docetaxel-refractory oesophagogastric cancer: the AGITG ATTAX2 trial. [Br J Cancer.](http://www.ncbi.nlm.nih.gov/pubmed/23412099) 2013 Mar 5;108(4):771-4.

[58. Heinemann V](http://www.ncbi.nlm.nih.gov/pubmed?term=Heinemann%20V%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23375249), [Douillard JY](http://www.ncbi.nlm.nih.gov/pubmed?term=Douillard%20JY%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23375249), [Ducreux M](http://www.ncbi.nlm.nih.gov/pubmed?term=Ducreux%20M%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23375249), [Peeters M](http://www.ncbi.nlm.nih.gov/pubmed?term=Peeters%20M%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23375249). Targeted therapy in metastatic colorectal cancer -An example of personalised medicine in action. [Cancer Treat Rev.](http://www.ncbi.nlm.nih.gov/pubmed/23375249) 2013. pii: S0305-7372(13) 00006-6

[59. Johannessen CM](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Johannessen%20CM%22%5BAuthor%5D), [Boehm JS](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Boehm%20JS%22%5BAuthor%5D), [Kim SY](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Kim%20SY%22%5BAuthor%5D), [Thomas SR](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Thomas%20SR%22%5BAuthor%5D), [Wardwell L](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Wardwell%20L%22%5BAuthor%5D), [Johnson LA](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Johnson%20LA%22%5BAuthor%5D), [Emery CM](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Emery%20CM%22%5BAuthor%5D), [Stransky N](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Stransky%20N%22%5BAuthor%5D), [Cogdill AP](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Cogdill%20AP%22%5BAuthor%5D), [Barretina J](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Barretina%20J%22%5BAuthor%5D), [Caponigro G](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Caponigro%20G%22%5BAuthor%5D), [Hieronymus H](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Hieronymus%20H%22%5BAuthor%5D), [Murray RR](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Murray%20RR%22%5BAuthor%5D), [Salehi-Ashtiani K](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Salehi-Ashtiani%20K%22%5BAuthor%5D), [Hill DE](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Hill%20DE%22%5BAuthor%5D), [Vidal M](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Vidal%20M%22%5BAuthor%5D), [Zhao JJ](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Zhao%20JJ%22%5BAuthor%5D), [Yang X](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Yang%20X%22%5BAuthor%5D), [Alkan O](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Alkan%20O%22%5BAuthor%5D), [Kim S](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Kim%20S%22%5BAuthor%5D), [Harris JL](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Harris%20JL%22%5BAuthor%5D), [Wilson CJ](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Wilson%20CJ%22%5BAuthor%5D), [Myer VE](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Myer%20VE%22%5BAuthor%5D), [Finan PM](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Finan%20PM%22%5BAuthor%5D), [Root DE](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Root%20DE%22%5BAuthor%5D), [Roberts TM](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Roberts%20TM%22%5BAuthor%5D), [Golub T](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Golub%20T%22%5BAuthor%5D), [Flaherty KT](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Flaherty%20KT%22%5BAuthor%5D), [Dummer R](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Dummer%20R%22%5BAuthor%5D), [Weber BL](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Weber%20BL%22%5BAuthor%5D), [Sellers WR](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Sellers%20WR%22%5BAuthor%5D), [Schlegel R](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Schlegel%20R%22%5BAuthor%5D), [Wargo JA](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Wargo%20JA%22%5BAuthor%5D), [Hahn WC](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Hahn%20WC%22%5BAuthor%5D), [Garraway LA](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Garraway%20LA%22%5BAuthor%5D). COT drives resistance to RAF inhibition through MAP kinase pathway reactivation. Nature. 2010.468(7326)**,** 968–972.

[60. Villanueva J](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Villanueva%20J%22%5BAuthor%5D), [Vultur A](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Vultur%20A%22%5BAuthor%5D), [Lee JT](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Lee%20JT%22%5BAuthor%5D), [Somasundaram R](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Somasundaram%20R%22%5BAuthor%5D), [Fukunaga-Kalabis M](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Fukunaga-Kalabis%20M%22%5BAuthor%5D), [Cipolla AK](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Cipolla%20AK%22%5BAuthor%5D), [Wubbenhorst B](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Wubbenhorst%20B%22%5BAuthor%5D), [Xu X](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Xu%20X%22%5BAuthor%5D), [Gimotty PA](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Gimotty%20PA%22%5BAuthor%5D), [Kee D](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Kee%20D%22%5BAuthor%5D), [Santiago-Walker AE](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Santiago-Walker%20AE%22%5BAuthor%5D), [Letrero R](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Letrero%20R%22%5BAuthor%5D), [D'Andrea K](http://www.ncbi.nlm.nih.gov/pubmed?term=%22D%27Andrea%20K%22%5BAuthor%5D), [Pushparajan A](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Pushparajan%20A%22%5BAuthor%5D), [Hayden JE](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Hayden%20JE%22%5BAuthor%5D), [Brown KD](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Brown%20KD%22%5BAuthor%5D), [Laquerre S](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Laquerre%20S%22%5BAuthor%5D), [McArthur GA](http://www.ncbi.nlm.nih.gov/pubmed?term=%22McArthur%20GA%22%5BAuthor%5D), [Sosman JA](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Sosman%20JA%22%5BAuthor%5D), [Nathanson KL](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Nathanson%20KL%22%5BAuthor%5D), [Herlyn M](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Herlyn%20M%22%5BAuthor%5D). [Acquired resistance to BRAF inhibitors mediated by a RAF kinase switch in melanoma can be overcome by cotargeting MEK and IGF-1R/PI3K.](http://www.ncbi.nlm.nih.gov/pubmed/21156289) Cancer Cell. 2010.18(6), 683-695.

61. Kwiatkowski N, Jelluma N, Filippakopoulos P, Soundararajan M, Manak MS, Kwon M, Choi HG, Sim T, Deveraux QL, Rottmann S, Pellman D, Shah JV, Kops GJ, Knapp S, Gray NS. Small-molecule kinase inhibitors provide insight into Mps1 cell

Cycle function. Nat Chem Biol. 2010**.** 6(5),359-68.

62. Colombo R, Caldarelli M, Mennecozzi M, Giorgini ML, Sola F, Cappella P, Perrera C, Depaolini SR, Rusconi L, Cucchi U, Avanzi N, Bertrand JA, Bossi RT, Pesenti E, Galvani A, Isacchi A, Colotta F, Donati D, Moll J. Targeting the mitotic checkpoint for cancer therapy with NMS-P715, an inhibitor of MPS1 kinase. Cancer Res. 2010. 70(24),10255-64.

**第六章前期研究基础**

**6.1** **Mps1基因对表达B-RAFV600E的细胞中心体复制及纺锤体结构的影响**

免疫荧光法分析分别计数表达BRAFV600E的细胞在分裂间期Mps1基因未敲除组与Mps1基因敲除组中心体数目，发现Mps1基因敲除组与未敲除组出现3个以上中心体的细胞比例分别为6%、30%，Mps1 基因敲除组显著降低。提示

Mps1基因是BRAFV600E导致中心体复制异常必不可少的下游靶基因。



**Figure 1** BRAFV600E induces centrosome amplification and multipolar spindles at least in part through Mps1-dependent defects in centrosome duplication in Sbcl2 cells. A-F**,** Sbcl2 pBabe-BRAFV600E cells were infected with pSUPER or pSUPER-Mps1 retrovirus. **A**, Forty eight hours post-infection, cells were subjected to Western blot to show the efficacy of Mps1 depletion. **B,** Cells described in A were fixed and subjected to IF by using monoclonal N1 anti-Mps1 antibody to show the efficacy and

Specificity of Mps1 depletion. **C,** Quantification of spindle abnormalities from the cells in B. **D,** Quantification of centrosomes (as judged byγ-tubulin foci) in BRAFV600E-expressing cells with or without Mps1 depletion. **E,** Cells described in A were fixed and subjected to IF by using anti-γand -α-tublin antibodies to show centrosomes and spindles respectively. **F,** BRAFV600E -expressing Sbcl2 or SK-MEL31 cells were arrested in S-phase with a 24-hr HU treatment, then infected with pSUPER. retro or pSUPER. retro-Mps1 retrovirus. Centrosome number was determined after an additional 48 hr of S-phase arrest. Bar graph shows the percentage of cells with three or more centrosomes.



**Figure 2. BRAFV600E induces centrosome amplification and multipolar spindles t at least in part through Mps1-dependent defects in centrosome duplication in SK-MEL31 cells. A,** SK-MEL31 cells were transfected with pEBG-GST vector or pEBG-GST-BRAFV600E. 24 hours

Post transfection, cells were infected with pSUPER. retro or pSUPER. retro-Mps1 retrovirus and subjected to Western blot showed the efficacy of Mps1 depletion. Actin was used as loading control. **B,** Cells described in A were fixed and subjected to IF by using monoclonal N1 anti-Mps1 antibody to show the efficacy and specificity of Mps1 depletion. **C,** Bar graph shows that quantization of spindle abnormality in B cells. At least 200 mitotic cells were assessed. Three independent experiments were performed. **D,** Quantification of centrosomes (as judged by

γ-tublin foci） in BRAFV600E-expressing cells with or without Mps1 depletion. Roughly 200

Interphase cells were assessed at each treatment. Three independent experiments were performed. **E,** Cells described in A were fixed and subjected to IF with anti-γand–α-tublin antibodies to show centrosomes and spindles respectively.

**6.2** **Mps1蛋白上B-RAFV600E特异性磷酸化位点的鉴定**

前期利用LC-MS\MS利用MEK抑制剂U0126进一步鉴定出Mps1上B-RAFV600E的特异性磷酸化作用靶点。



Figure 2**. Identification of BRAFV600E-dependent phosphorylated sites on human Mps1 protein by LC-MS\MS. A,** Mps1 is hyperphosphorylated in BRAFV600E-expressing cells. 293T cells were co-transfected with pEBG-GST-BRAFV600E and pHF36-GFP-Mps1 wild-type plasmids. Twenty four hours post transfection, the MEK1/2 specific inhibitor U0126 was added to cell

Cultures for 6 hours (10-20 mM final) to block MAPK activation. Cell lysates were immunoprecipitated with anti-GFP monoclonal antibody. GFP-Mps1 immunocomplexes were then incubated in a phosphatase buffer (50mM Tris-HCl pH 8.0, 0.1 mM ZnCl2, and 1 mM MgCl2) supplemented with or without 10U of lambda protein phosphatase (λ-PPase) for 30 min at 37ºC, and analyzed by immunoblotting. Phospho-ERK, CAS, and BRAF were analyzed by immunoblotting in cell lysates. **B,** Mps1 immunocomplexes were subjected to silver staining. Red box shows the Mps1 band of interest, which was cut out and subjected to LC-MS/MS analysis. **C,** Representative data from the LC-MS/MS analysis of Mps1 that was

Immunprecipitated from BRAFV600E-expressing cells in the presence or absence of

U0126 as described in Figure S3B. **D,** Alignment of vertebrate Mps1 proteins showing that the MAPK phosphorylated sites are conserved in human, mouse, and Xenopus. **E,** Summary of different mutations in Mps1. Mps1 mutants were constructed into pBabe-HA-puro, pBabe-GFP-puro, and pEF36-HA vectors followed the QuikChange®Site-Directed Mutagenesis Kit instruction manual (Stratagene) and

Verified by sequencing.

**6.3** **B-RAFV600E相应位点磷酸化对Mps1与Cdc27结合能力的影响**

Cdc27是泛素连接酶APC/C的亚单位，首先将Sbcl2细胞过表达WT-Mps1, HA-Mps1, 24h后收集细胞以anti-HA或anti-Cdc27进行免疫共沉淀实验，最后样品进行Western bloting检测对Mps1与Cdc27相互作用的影响。结果表明，过表达WT-Mps1、S436A/E、S821A/E细胞中，在Mps1与Cdc27免疫沉淀复合物中分别检测到Cdc27与Mps1，过表达S281E细胞中，Mps1与Cdc27无相互作用。提示pS281-Mps1抑制Mps1与Cdc27的相互作用。紧接着我们验证了pS281-Mps1对Mps1蛋白泛素化水平的影响。如图所示：与S281A、S436A/E及S821A/E三组相比，S281E显著抑制Mps1泛素化水平。综上研究表明，pS281-Mps1通过阻止Mps1与Cdc27结合而抑制Mps1蛋白的泛素化作用。



**Figure** **3** Phosphor ylation of S281 residue, disrupts the interac tion between Mps1 and Cdc27, and is impor tant for Mps1 stabilization

# 综 述

**癌基因B-RAFV600E在肿瘤发Th发展中的研究进展**

丝裂原活化蛋白激酶(mitogen．Activated protein kinases, MAPKs)是细胞内的类丝氨酸/苏氨酸蛋白激酶。MAPKs信号转导通路存在于大多数细胞内，在将细胞外刺激信号转导至细胞及其核内，并在引起细胞增殖、分化、转化及凋亡等生物学反应的过程中具有至关重要的作用[1-4]. RAF是一种丝/苏氨酸激酶，是

MAPK信号传导通路中RAS的下游作用底物、MEK的上游激酶，在哺乳动物中由3个家族成员组成，分别是A-RAF、B-RAF、C-RAF[5]。自从2002年Davies[6]等发现约70%恶性黑色素瘤和15%结直肠癌中存在体细胞B-RAF基因突变以来，科研人员近年来科研人员在人类许多肿瘤组织中都发现B-RAF基因的突变[7-17]。目前，已经有超过70个错义突变被证实，其中大约90%的点突变为T1796A，该点突变导致缬氨酸被谷氨酸所代替(V600E)，称为B-RAFV600E。可见，B-RAFV600E是恶性肿瘤的发生发展中最为常见的遗传变异事件。现就癌基因B-RAFV600E恶性肿瘤的发生发展中的研究进展做如下综述。

**1. B-RAF基因及突变类型**

B-RAF基因是1988年Ikawa[18]等学者在人类尤文氏肉瘤中发现一个能转染NIH3T3细胞且具有活性的DNA序列，由于其序列与C-RAF和A-RAF基因序列具有相当高的同源性，故命名为B-RAF基因。B-RAF，又名鼠类肉瘤滤过性毒菌致癌同源体Bl，位于人染色体7q34，含18个外显子，编码蛋白质分子全长约94 KD，有783个氨基酸残基，属于丝氨酸/苏氨酸蛋白质激酶类，该蛋白包括3个保守区域：CR1、CR2和CR3[19-21]。其中CR1包含RAS结合域（RAS-binding domain, RBD）和富含半胱氨酸结构域（cysteine-rich domain，

CRD），CR2 为氨基末端调节域，CR3 是其激酶所在区域（图1）。该基因含有至少7个转录区，可以编码多种蛋白质，其中包括B型有丝分裂原激活的蛋白

激酶依赖性激酶，属于丝氨酸/苏氨酸蛋白激酶类[19]，参与RAS-RAF-有丝分裂原活化蛋白/细胞外信号调节激酶（MEK）-细胞外信号调节激酶（ERK）-丝裂原活化蛋白激酶（MAPK）途径的信号传导，在细胞增殖、分化、转化及凋亡等生物学反应过程中具有至关重要的作用[1-4]。

与A-RAF和C-RAF很少发生基因突变不同的是，B-RAF基因突变是人体肿瘤中最常见的一种RAF基因突变方式。2002年, Davies等[6]对530个肿瘤细胞株基因组DNA进行突变筛选，指出B-RAF基因在60%以上的恶性黑色素瘤及少数结直肠癌、甲状腺癌等肿瘤中都有突变。目前已经发现有超过45种B-RAF基因突变类型，其中包括B-RAFV600E，B-RAFK601E，AKAP9-B-RAF，B-RAFV599ins, B-RAFV600E+K601del和B-RAFV600D+FGLAT601-605ins等[22-26]。但其中大约

90%的点突变为T1796A，该点突变导致缬氨酸被谷氨酸所代替(V600E)，称为B-RAFV600E。



**图1** **B-RAF基因编码蛋白示意图（由Kam-Tsun Tang 提供）**

**2. B-RAFV600E 在各种肿瘤中的突变频率与临床分级、预后的关系**

自从2002年Davies[6]等发现约70%恶性黑色素瘤和15%结直肠癌中存在体细胞B-RAF基因突变以来，科研人员已在人类许多肿瘤组织中都发现B-RAF基因的突变，国外人群中70%的皮肤癌[7]，50%的甲状腺癌[8]，20%的结肠癌[9]，14-30%的卵巢癌[10]，15%的肝癌[11]，5%的肺癌、乳腺癌中都检测到B-RAF基因的突变[12-13]. B-RAF基因在中国人非肢端皮肤黑色素瘤中突变频率高达43.3%

（13/30），且以V600E 突变为主[14]。而该基因在中国人结直肠癌、甲状腺癌中的突变频率因样本量小而报道不一，分别约26%-65%、25-50%[15-17]. YuenST 等

[27]已证实B-RAF蛋白在高、中、低分化结直肠癌中的表达阳性率差异有显著性意义，且B-RAF统计学上的表达模式符合“增生性息肉-锯齿状腺瘤-癌”递增趋势。朱琰琰等[28]的研究还发现, B-RAF基因的突变状态与恶性黑色素瘤的分期、预后有关，存在B-RAF突变者预后不良的现象。有研究发现[29-30] BRAF基因突变是甲状腺癌一个有力的风险预后指标，术前甲状腺细针穿刺活检(FNAB)检测到B-RAF基因突变与临床病理结果较差的甲状腺乳头状癌标本显著相关[32-33]。

Xing[30]等报道219例甲状腺乳头状癌B-RAF基因突变与甲状腺癌侵袭性生物学行为相关，表现为癌组织的包膜外扩散、局部淋巴结转移及较高的临床复发率。



**图2** **MAPK信号通路中RAF突变**

**3. B-RAFV600E 在肿瘤发Th发展中作用机制的研究**

人们已经在结肠息肉和良性黑色素瘤中发现B-RAF基因的突变，说明该基因突变是肿瘤发生发展过程中的早期事件[31]。Green MR等发现癌基因B-RAFV600E通过IGFBP7途径诱导细胞衰老和凋亡发生[32]。Yamashita等报道B-RAFV600E在甲状腺癌中可以激活NF-κB信号通路[33]，并且可能与p53有关[34]。崔永萍等首次发现了B-RAF基因在有丝分裂期的新作用[35]，该基因突变后可导

致人皮肤癌细胞、黑色素细胞和永生化上皮细胞出现纺锤体异常、染色体不稳定性及非整倍体细胞出现[36]。Riesco等[37]发现B-RAFV600E突变能诱导TGF2β的分泌，而TGF2β能抑制Na+/ I-同向转运体(NIS)基因的功能。最终导致无法通过手术治疗的患者对放射性碘治疗不敏感，从而增加了甲状腺癌的病死率。

该基因在体外激酶活性是野生型的500 倍，可持续、失控激活的

RAF-MEK-ERK信号通路，影响多种恶性肿瘤相关基因的表达，包括CyclinD、

VEGF、C-myc、β3-integrin等表达增加和原凋亡蛋白BIM家族表达下降，导致细胞过度增殖、分化，从而在肿瘤发生发展中发挥重要作用[38-41]（图3）。另外，研究报道B-RAF基因与K-RAS基因虽然同处RAS-RAF-MAPK通路，但两者突变具有相互排它性，只有大约1%肿瘤同时存在RAS和B-RAF基因的突变[42]，因此，B-RAF基因能不依赖于K-RAS基因而独立参与肿瘤的发生发展过程。裸鼠实验表明，在NIH3T3细胞中B-RAFV600E过度活化ERK，诱导细胞增殖、转化，在裸鼠体内产生肿瘤[43]。近年来科学家又发现了B-RAF基因在有丝分裂期的新作用，该基因参与细胞有丝分裂期纺锤体形成、染色体分离、纺锤体检测点功能调控，在维持细胞染色体稳定性中发挥重要功能[44]；癌基因B-RAFV600E可导致纺锤体结构及定位异常、染色体不稳定性等有丝分裂期异常现象，在肿瘤细胞染色体不稳定性及非整倍体产生中发挥重要作用[45-46]。本课题组进一步经质谱分析又鉴定出癌基因B-RAFV600E新的作用底物—纺锤体蛋白激酶Mps1，二者在有丝分裂期均定位于着丝粒和中心体处，B-RAFV600E主要通过增强Mps1的稳定性而使Mps1蛋白水平以及激酶活性显著增加，导致纺锤体检测点过度激活。进一步机制研究发现，癌基因B-RAFV600E通过磷酸化修饰纺锤体监测点激酶Mps1-S281、S436、S821，分别影响Mps1激酶的蛋白质稳定性、激酶活性、着丝粒定位能力等[46]。因此，B-RAFV600E被公认为一个非常重要的癌基因。



**图3** **MAPK信号通路中RAF突变（由Carmelo Nucera提供）**

**3. 针对B-RAFV600E的各种肿瘤治疗策略**

近年来肿瘤分子靶向治疗发展迅速，B-RAF基因在人类多种肿瘤组织中发生不同比例的突变，国外人群中在皮肤癌、甲状腺癌、结肠癌等恶性肿瘤中突变频率较高[47-49]。在中国人非肢端皮肤黑色素瘤中突变频率高达43.3%（13/30），且以V600E突变为主[50]。而该基因在中国人结直肠癌、甲状腺癌中的突变频率因样本量小而报道不一，分别约26%-65%、25-50%[15-17]。可见，B-RAF基因突变是目前人类多种恶性肿瘤中最为常见的基因变异，有可能成为靶向药物作用的靶点。

以结直肠癌为例，抗EGFR靶向治疗为结直肠癌患者带来了希望。多项临床研究表明，只有部分结直肠癌患者对抗EGFR靶向治疗有效如西昔妥单抗

（Cetuximab, Erbitux, C225, 爱必妥）竟争性与EGFR的胞外激酶特异性结合，并且抑制EGFR与其它配体的结合，从而抑制下游信号通路的传导达到抗肿瘤的作用[51-53]。但在EGFR表达阳性的患者中C225的反应率仅能达到10%，最近的研究表明EGFR的表达、EGFR的突变状况并不能作为预测C225疗效的生物学标志

[54-55]。尽管K-RAS基因突变已经作为抗EGFR靶向治疗的一个预测指标，但并不

是所有K-RAS野生型患者都对该靶向治疗有效。现除KRAS外仍无一公认的生物学标志物来预测C225的疗效，所以仍需发现新的生物学标志来预测单抗的疗效。研究报道B-RAF基因与K-RAS基因虽然同处RAS-RAF-MAPK通路，但两者突变具有相互排它性，只有大约1%肿瘤同时存在RAS和B-RAF基因的突变[42]，因此，B-RAF基因能不依赖于K-RAS基因而独立参与肿瘤的发生发展过程，有可能成为靶向药物作用的靶点。多项研究表明K-RAS野生型而B-RAFV600E突变型患者对

EGFR单克隆抗体原发耐药，其无进展生存期和总生存率较B-RAF野生型都较短。B-RAF基因型与抗EGFR靶向治疗的疗效及预后密切相关，是抗EGFR靶向治疗的一个独立影响因素[56-57]。因此，迫切需要抗EGFR靶向治疗联合一些选择性B-RAF抑制剂（如PLX-4032和XL-281）的肿瘤治疗策略。

目前一些针对B-RAF激酶特异抑制剂已被成功应用于临床癌症治疗，例如BAY43-9006是RAF激酶特异性抑制剂，其在携带B-RAFV600E突变体黑色素瘤患者的临床治疗中显示显著疗效，但同时发现部分病人存在严重的BAY43-9006耐药性问题，甚至促进了肿瘤的发生发展[58-59]。进一步机制研究发现，尽管B-RAF特异性抑制剂可以抑制携带B-RAFV600E突变体细胞中MEK/ERK信号通路，但是该抑制剂反而可激活携带K-RAS基因突变及B-RAF野生型细胞中MEK/ERK通路。在这些细胞中由于B-RAF/C-RAF、C-RAF/C-RAF或B-RAF-KD/C-RAF等二聚体的形成，反而使MEK/ERK通路活性更强，从而促进肿瘤细胞增殖[60]。而且，

RAF抑制剂不仅仅过渡激活携带K-RAS基因突变细胞的MEK/ERK通路，对于那

些被其它癌基因（如HER2）激活的MEK/ERK通路，同样具有过度激活的作用

[61-62]. 另外，有报道COT基因[63]、IGF-1R/PI3K[64]途径等可能与RAF抑制剂耐药

性有关。尽管RAF激酶抑制剂在体外细胞水平的耐药机制研究2010年以来有所突破，但目前仍缺乏有效策略，尚需系统深入研究癌基因B-RAFV600E在肿瘤发生发展中的作用机制，并从中鉴定新的靶标基因，这对筛选新的药物靶点、指导临床联合使用药物策略有重要意义。

本研究发现癌基因B-RAFV600E通过磷酸化修饰有丝纺锤体监测点激酶Mps1-S281、S436、S821，分别影响Mps1激酶的蛋白质稳定性、激酶活性、着丝粒定位能力等，进而导致中心体过渡复制、多级纺锤体出现，在肿瘤细胞染色体不稳定性、非整倍体细胞形成中发挥重要作用[46]，提示Mps1可能作为携带B-RAFV600E突变型恶性肿瘤新的治疗靶点。

**4. 小结与展望**

综上所述，癌基因B-RAFV600E是多种恶性肿瘤中最为常见的基因变异，在肿瘤的发生发展中发挥着至关重要的作用，有可能成为靶向药物作用的靶点。虽然目前临床上已经使用了一些B-RAFV600E抑制剂如BAY43-9006，但其效果并不理想。未来，仍需深入探讨B-RAFV600E在恶性肿瘤发生发展中的作用机制。如除V600E突变外，其它类型的突变是否具有相应的生物学效应；在细胞周期调控过程中，是否象其它癌基因一样，需要“二次打击”等这些问题仍有待进一步论证。另外，我们的研究发现癌基因B-RAFV600E通过磷酸化修饰有丝纺锤体监测点激酶Mps1-S281、S436、S821，分别影响Mps1激酶的蛋白质稳定性、激酶活性、着丝粒定位能力等，进而导致中心体过渡复制、多级纺锤体出现，在肿瘤细胞染色体不稳定性、非整倍体细胞形成中发挥重要作用[46]，提示Mps1可能作为携带B-RAFV600E突变型恶性肿瘤新的治疗靶点。那么，我们需要深入B-RAFV600E和Mps1在恶性肿瘤发生发展中相关性、作用机制，研究对携带B-RAFV600E突变型恶性肿瘤新治疗的可能性，为携带B-RAFV600E突变型恶性肿瘤的临床治疗提供理论依据。

参考文献：

[1]. Weisbart RH, Chan G, Li E, Farmani N, Heinze E, Rubell A, Nishimura RN, Colburn K. [BRAF splice variants in rheumatoid arthritis synovial fibroblasts activate MAPK through CRAF.](http://www.ncbi.nlm.nih.gov/pubmed/23517740) Mol Immunol. 2013, S0161-5890(13) 00039-4.

[[2]. Zhou Y](http://www.ncbi.nlm.nih.gov/pubmed?term=Zhou%20Y%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23501044), [Zhang M](http://www.ncbi.nlm.nih.gov/pubmed?term=Zhang%20M%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23501044), [Sun GY](http://www.ncbi.nlm.nih.gov/pubmed?term=Sun%20GY%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23501044), [Liu YP](http://www.ncbi.nlm.nih.gov/pubmed?term=Liu%20YP%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23501044), [Ran WZ](http://www.ncbi.nlm.nih.gov/pubmed?term=Ran%20WZ%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23501044), [Peng L](http://www.ncbi.nlm.nih.gov/pubmed?term=Peng%20L%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23501044), [Guan CX](http://www.ncbi.nlm.nih.gov/pubmed?term=Guan%20CX%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23501044). Calcitonin gene-related peptide promotes the wound healing of human bronchial epithelial cells via PKC andMAPK pathways. [Regul Pept.](http://www.ncbi.nlm.nih.gov/pubmed/23501044) 2013, Mar 14. pii: S0167-0115(13) 00049-9.

[3]. Fang JQ, Du JY, Liang Y, Fang JF. [Intervention of electroacupuncture on spinal p38 MAPK/ATF-2/VR-1 pathway in treating inflammatory pain induced by CFA in rats.](http://www.ncbi.nlm.nih.gov/pubmed/23517865) Mol Pain. 2013, 9(1): 13. [Epub ahead of print].

[4]. Robert G, Jullian V, Jacquel A, Ginet C, Dufies M, Torino S, Pottier A, Peyrade F, Tartare-Deckert S, Bourdy G, Deharo E, Auberger P. [Simalikalactone E (SkE), a new weapon in the armamentarium of drugs targeting cancers that exhibit constitutive activation of the ERK pathway.](http://www.ncbi.nlm.nih.gov/pubmed/23518796) Oncotarget. 2012, 3(12): 1688-9.

[5]. Brummer T, Martin P, Reth M. Functional analysis of the regulatory requi rements of B-Raf and the B-Raf(V600E) oncoprotein. Oncogene. 2006, 25(47): 6262-76.

[6]. Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF gene in human cancer. Nature, 2002, 417: 949- 954.

[7]. Dumaz N. Mechanism of RAF isoform switching induced by oncogenic RAS in melanoma. Small Gtpases. 2011, 2(5), 289-292.

[8]. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, Davis N, Dicks E, Ewing R, Floyd Y, Gray K, Hall S,

Hawes R, Hughes J, Kosmidou V, Menzies A, Mould C, Parker A, Stevens C, Watt S, Hooper S, Wilson R, Jayatilake H, Gusterson BA, Cooper C, Shipley J, Hargrave D, Pritchard–Jones K, Maitland N, Chenevix-Trench G, Rigins GL, Bigner DD, Palmieri G, Cossu A, Flanagan A, Nicholson A, Ho JW, Leung SY, Yuen ST, Weber BL, Seigler HF, Darrow TL, Paterson H, Marais R, Marshall CJ, Wooster R, Stratton MR, Futreal PA. Mutations of the BRAF gene in human cancer. Nature. 2002, 417(6892), 949–954.

[[9]. Anderson S](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Anderson%20S%22%5BAuthor%5D), [Bloom KJ](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Bloom%20KJ%22%5BAuthor%5D), [Vallera DU](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Vallera%20DU%22%5BAuthor%5D), [Rueschoff J](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Rueschoff%20J%22%5BAuthor%5D), [Meldrum C](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Meldrum%20C%22%5BAuthor%5D), [Schilling R](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Schilling%20R%22%5BAuthor%5D), [Kovach B](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Kovach%20B%22%5BAuthor%5D), [Lee JR](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Lee%20JR%22%5BAuthor%5D), [Ochoa P](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Ochoa%20P%22%5BAuthor%5D), [Langland R](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Langland%20R%22%5BAuthor%5D), [Halait H](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Halait%20H%22%5BAuthor%5D), [Lawrence HJ](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Lawrence%20HJ%22%5BAuthor%5D), [Dugan MC](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Dugan%20MC%22%5BAuthor%5D). Multisite Analytic Performance Studies of a Real-Time Polymerase Chain Reaction Assay for the Detection of BRAF V600E Mutations in Formalin-Fixed Paraffin-Embedded Tissue Specimens of Malignant Melanoma. [Arch Pathol Lab Med.](http://www.ncbi.nlm.nih.gov/pubmed/22332713#%23) 2012. [Epub ahead of print]

[[10]. Kim SJ](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Kim%20SJ%22%5BAuthor%5D), [Lee KE](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Lee%20KE%22%5BAuthor%5D), [Myong JP](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Myong%20JP%22%5BAuthor%5D), [Park JH](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Park%20JH%22%5BAuthor%5D), [Jeon YK](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Jeon%20YK%22%5BAuthor%5D), [Min HS](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Min%20HS%22%5BAuthor%5D), [Park SY](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Park%20SY%22%5BAuthor%5D), [Jung KC](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Jung%20KC%22%5BAuthor%5D), [Koo do H](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Koo%20do%20H%22%5BAuthor%5D), [Youn YK](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Youn%20YK%22%5BAuthor%5D). BRAF(V600E) Mutation is Associated with Tumor Aggressiveness in Papillary Thyroid Cancer. [World J Surg.](http://www.ncbi.nlm.nih.gov/pubmed/22190222#%23) 2012, 36(2): 310-7.

[[11]. Kalady MF](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Kalady%20MF%22%5BAuthor%5D), [Dejulius KL](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Dejulius%20KL%22%5BAuthor%5D), [Sanchez JA](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Sanchez%20JA%22%5BAuthor%5D), [Jarrar A](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Jarrar%20A%22%5BAuthor%5D), [Liu X](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Liu%20X%22%5BAuthor%5D), [Manilich E](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Manilich%20E%22%5BAuthor%5D), [Skacel M](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Skacel%20M%22%5BAuthor%5D), [Church JM](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Church%20JM%22%5BAuthor%5D). BRAF mutations in colorectal cancer are associated with distinct clinical characteristics and worse prognosis. [Dis Colon Rectum.](http://www.ncbi.nlm.nih.gov/pubmed/22228154) 2012, 55(2), 128-33.

[[12]. Wong KK](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Wong%20KK%22%5BAuthor%5D), [Tsang YT](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Tsang%20YT%22%5BAuthor%5D), [Deavers MT](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Deavers%20MT%22%5BAuthor%5D), [Mok SC](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Mok%20SC%22%5BAuthor%5D), [Zu Z](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Zu%20Z%22%5BAuthor%5D), [Sun C](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Sun%20C%22%5BAuthor%5D), [Malpica A](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Malpica%20A%22%5BAuthor%5D), [Wolf JK](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Wolf%20JK%22%5BAuthor%5D), [Lu KH](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Lu%20KH%22%5BAuthor%5D), [Gershenson DM](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Gershenson%20DM%22%5BAuthor%5D). BRAF mutation is rare in advanced-stage low-grade ovarian serous carcinomas. [Am J Pathol.](http://www.ncbi.nlm.nih.gov/pubmed/20802181#%23) 2010, 177(4), 1611-7.

[[13]. Colombino M](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Colombino%20M%22%5BAuthor%5D), [Sperlongano P](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Sperlongano%20P%22%5BAuthor%5D), [Izzo F](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Izzo%20F%22%5BAuthor%5D), [Tatangelo F](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Tatangelo%20F%22%5BAuthor%5D), [Botti G](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Botti%20G%22%5BAuthor%5D), [Lombardi A](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Lombardi%20A%22%5BAuthor%5D), [Accardo M](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Accardo%20M%22%5BAuthor%5D), [Tarantino L](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Tarantino%20L%22%5BAuthor%5D), [Sordelli I](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Sordelli%20I%22%5BAuthor%5D), [Agresti M](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Agresti%20M%22%5BAuthor%5D), [Abbruzzese A](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Abbruzzese%20A%22%5BAuthor%5D), [Caraglia M](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Caraglia%20M%22%5BAuthor%5D), [Palmieri G](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Palmieri%20G%22%5BAuthor%5D). BRAF and PIK3CA genes are somatically mutated in hepatocellular carcinoma among

Patients from South Italy[. Cell Death Dis.](http://www.ncbi.nlm.nih.gov/pubmed/22258409#%23) 2012, 3: e259

[[14]. Kobayashi M](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Kobayashi%20M%22%5BAuthor%5D), [Sonobe M](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Sonobe%20M%22%5BAuthor%5D), [Takahashi T](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Takahashi%20T%22%5BAuthor%5D), [Yoshizawa A](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Yoshizawa%20A%22%5BAuthor%5D), [Ishikawa M](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Ishikawa%20M%22%5BAuthor%5D), [Kikuchi R](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Kikuchi%20R%22%5BAuthor%5D), [Okubo K](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Okubo%20K%22%5BAuthor%5D), [Huang CL](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Huang%20CL%22%5BAuthor%5D), [Date H](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Date%20H%22%5BAuthor%5D). Clinical significance of BRAF gene mutations in patients with non-small cell lung cancer. [Anticancer Res.](http://www.ncbi.nlm.nih.gov/pubmed/22199339#%23) 2011, 31(12), 4619-23.

[15. Greenman C](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Greenman%20C%22%5BAuthor%5D), [Stephens P](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Stephens%20P%22%5BAuthor%5D), [Smith R](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Smith%20R%22%5BAuthor%5D), [Dalgliesh GL](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Dalgliesh%20GL%22%5BAuthor%5D), [Hunter C](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Hunter%20C%22%5BAuthor%5D), [Bignell G](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Bignell%20G%22%5BAuthor%5D), [Davies H](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Davies%20H%22%5BAuthor%5D), [Teague J](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Teague%20J%22%5BAuthor%5D), [Butler A](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Butler%20A%22%5BAuthor%5D), [Stevens C](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Stevens%20C%22%5BAuthor%5D), [Edkins S](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Edkins%20S%22%5BAuthor%5D), [O'Meara S](http://www.ncbi.nlm.nih.gov/pubmed?term=%22O%27Meara%20S%22%5BAuthor%5D), [Vastrik I](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Vastrik%20I%22%5BAuthor%5D), [Schmidt EE](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Schmidt%20EE%22%5BAuthor%5D), [Avis T](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Avis%20T%22%5BAuthor%5D), [Barthorpe S](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Barthorpe%20S%22%5BAuthor%5D), [Bhamra G](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Bhamra%20G%22%5BAuthor%5D), [Buck G](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Buck%20G%22%5BAuthor%5D), [Choudhury B](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Choudhury%20B%22%5BAuthor%5D), [Clements J](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Clements%20J%22%5BAuthor%5D), [Cole J](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Cole%20J%22%5BAuthor%5D), [Dicks E](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Dicks%20E%22%5BAuthor%5D), [Forbes S](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Forbes%20S%22%5BAuthor%5D), [Gray K](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Gray%20K%22%5BAuthor%5D), [Halliday K](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Halliday%20K%22%5BAuthor%5D), [Harrison R](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Harrison%20R%22%5BAuthor%5D), [Hills K](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Hills%20K%22%5BAuthor%5D), [Hinton J](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Hinton%20J%22%5BAuthor%5D), [Jenkinson A](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Jenkinson%20A%22%5BAuthor%5D), [Jones D](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Jones%20D%22%5BAuthor%5D), [Menzies A](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Menzies%20A%22%5BAuthor%5D), [Mironenko T](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Mironenko%20T%22%5BAuthor%5D), [Perry J](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Perry%20J%22%5BAuthor%5D), [Raine K](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Raine%20K%22%5BAuthor%5D), [Richardson D](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Richardson%20D%22%5BAuthor%5D), [Shepherd R](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Shepherd%20R%22%5BAuthor%5D), [Small A](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Small%20A%22%5BAuthor%5D), [Tofts C](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Tofts%20C%22%5BAuthor%5D), [Varian](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Varian%20J%22%5BAuthor%5D) J, [Webb T](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Webb%20T%22%5BAuthor%5D), [West S](http://www.ncbi.nlm.nih.gov/pubmed?term=%22West%20S%22%5BAuthor%5D), [Widaa S](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Widaa%20S%22%5BAuthor%5D), [Yates A](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Yates%20A%22%5BAuthor%5D), [Cahill DP](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Cahill%20DP%22%5BAuthor%5D), [Louis DN](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Louis%20DN%22%5BAuthor%5D), [Goldstraw P](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Goldstraw%20P%22%5BAuthor%5D), [Nicholson AG](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Nicholson%20AG%22%5BAuthor%5D), [Brasseur F](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Brasseur%20F%22%5BAuthor%5D), [Looijenga L](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Looijenga%20L%22%5BAuthor%5D), [Weber BL](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Weber%20BL%22%5BAuthor%5D), [Chiew YE](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Chiew%20YE%22%5BAuthor%5D), [DeFazio A](http://www.ncbi.nlm.nih.gov/pubmed?term=%22DeFazio%20A%22%5BAuthor%5D), [Greaves MF](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Greaves%20MF%22%5BAuthor%5D), [Green AR](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Green%20AR%22%5BAuthor%5D), [Campbell P](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Campbell%20P%22%5BAuthor%5D), [Birney E](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Birney%20E%22%5BAuthor%5D), [Easton DF](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Easton%20DF%22%5BAuthor%5D), [Chenevix-Trench G](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Chenevix-Trench%20G%22%5BAuthor%5D), [Tan MH](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Tan%20MH%22%5BAuthor%5D), [Khoo SK](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Khoo%20SK%22%5BAuthor%5D), [Teh BT](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Teh%20BT%22%5BAuthor%5D), [Yuen ST](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Yuen%20ST%22%5BAuthor%5D), [Leung SY](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Leung%20SY%22%5BAuthor%5D), [Wooster R](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Wooster%20R%22%5BAuthor%5D), [Futreal PA](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Futreal%20PA%22%5BAuthor%5D), [Stratton MR](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Stratton%20MR%22%5BAuthor%5D). Patterns of somatic mutation in human cancer genomes. [Nature.](http://www.ncbi.nlm.nih.gov/pubmed/17344846#%23) 2007, 446(7132), 153-8.

[16. Si L](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Si%20L%22%5BAuthor%5D), [Kong Y](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Kong%20Y%22%5BAuthor%5D), [Xu X](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Xu%20X%22%5BAuthor%5D), [Flaherty KT](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Flaherty%20KT%22%5BAuthor%5D), [Sheng X](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Sheng%20X%22%5BAuthor%5D), [Cui C](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Cui%20C%22%5BAuthor%5D), [Chi Z](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Chi%20Z%22%5BAuthor%5D), [Li S](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Li%20S%22%5BAuthor%5D), [Mao L](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Mao%20L%22%5BAuthor%5D), [Guo J](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Guo%20J%22%5BAuthor%5D). Prevalence of BRAF V600E mutation in Chinese melanoma patients: large scale analysis of BRAF and NRAS mutations in a 432-case cohort. [Eur J Cancer.](http://www.ncbi.nlm.nih.gov/pubmed/21788131#%23) 2012, 48(1), 94-100.

17. Liou JM, Wu MS, Shun CT, Chiu HM, Chen MJ, Chen CC, Wang HP, Lin JT, Liang JT. [Mutations in BRAF correlate with poor survival of colorectal cancers in Chinese population.](http://www.ncbi.nlm.nih.gov/pubmed/21553007) Int J Colorectal Dis. 2011, 26(11):1387-95.

18. Ikawa S, Fukui M, yama Y, et al. B2raf, a new member of theraf family, is activated by DNA rearrangement. Mol Cell Biol,1988, 8:2651 - 2654.

19. Zhang BH, Guan KL. Activation of B-Raf kinase requires phosphorylation of

Theconserved residues Thr598 and Ser601. EMBO J 2000, 19: 5429-39

20. Kolch W. Meaningful relationships: the regulation of the Ras/Raf/MEK/ERK pathway by protein interactions. Biochem J 2000, 351:289–305.

21. Leicht DT, Balan V, Kaplun A, et al. Raf kinases: function, regulation androle in human cancer. Biochim Biophys Acta 2007, 1773:1196–212.

22. Trovisco V, Vieira de Castro I, Soares P, et al. BRAF mutations are associated with

Some histological types of papillary thyroid carcinoma. Pathol 2004, 202:247–51.

23. Ciampi R, Knauf JA, Kerler R, Gandhi M, et al. Oncogenic AKAP9-BRAF fusion is a novel mechanism of MAPK pathway activation in thyroid cancer. Clin Invest

2005, 115:94–101.

24. Moretti S, Macchiarulo A, De Falco V, et al. Biochemical and molecular characterization of the novel BRAF (V599Ins) mutation detected in a classic papillary

Thyroid carcinoma. Oncogene 2006, 25:4235–40.

25. Hou P, Liu D, Xing M. Functional characterization of the T1799–1801del and A1799–1816ins BRAF mutations in papillary thyroid cancer. Cell Cycle 2007, 6:377–9.

26. Oler G, Ebina KN, Michaluart P Jr, et al. Investigation of BRAF mutation in a series of papillary thyroid carcinoma and matched-lymph node metastasis reveals a new mutation in metastasis. Clin Endocrinol (Oxf) 2005, 62:509–11.

27. Yuen ST, davies H, Chan TL, et a. l Similarity of the phenotypicpatterns associated with BRAF and KRASmutations incolorectal neoclassic. Cancer Res, 2004, 62:6451- 6455.

28. 朱琰琰，斯璐，迟志宏，等.中国黑色素瘤患者BRAF基因突变分析. 临床肿瘤学杂志, 2009, 14: 585-588.

29. XingM, ClarkD, GuanH, et al. BRAFmutation testingof thyroid fine2needle aspiration biopsy specimens for preoperative risk stratification in papillary thyroid cancer[ J ]. J Clin Oncol, 2009, 27(18): 2977 - 2982.

30. Marchetti I, Lessi F, Mazzanti CM, et al. Amorpho2molecular diagnosis of papillary thyroid carcinoma: BRAFV600E detection as an important tool in preoperative evaluation of fine2needle aspirates [ J ]. Thyroid, 2009, 19 ( 8): 837- 842.

31. Yazdi AS, Palmedo G, Flaig MJ. Mutations of the B-Raf gene in benign and malignant melanocytic lesions. J Invest Dermatol. 2003, 121: 1160-1162.

32. Wajapeyee N., Serra RW., Zhu X., Mahalingam M., Green MR. Oncogenic BRAF induces senescence and apoptosis through pathway mediated by the secreted protein IGFBP7. Cell. 2008, 132: 363-374.

33. Palona I, Namba H, Yamashita S. BRafV600E promotes invasiveness of thyroid cancer cells through nuclear factor kappaB activation. Endocrinology. 2006, 147: 56995-707.

34. Patton EE, Widlund HR, Zon LI. B-RAF mutations are sufficient to promote nevi formation and cooperate with p53 in the genesis of melanoma. Curr Biol. 2005, 15: 249-254.

35. Calistri D, Rengucci C, Seymour I, Amadori D. Mutation analysis of p53, K-ras, and BRAF gene in colorectal cancer progression. J Cell Physiol. 2005, 204: 484-488.

36. Cui Y, Guadagno TM. B-RafV600E signaling deregulates the mitotic spindle checkpoint through stabilizing Mps1 levels in melanoma cells. Oncogene. 2008, 27:

3122-3133.

37. Riesco EG, Rodríguez I, DeLVA, et al. TheBRAFV600E oncogene induces transforming growth factor beta secretion leading to sodium iodide symporter repression and increased malignancy in thyroid cancer [ J ]. Cancer Res, 2009, 69(21): 8317 - 8325.

38. Kumar R, Angelini S, Czene, K, et al. B-Raf mutations in metastatic melanoma: a possible association with clinical outcome. Clin Cancer Res. 2003, 9: 3362-3368.

39. Fukushima T, Suzuki S, Mashiko M, et al. B-Raf mutations in papillary carcinomas of the thyroid. Oncogene. 2003, 22: 64556457.

40. Nikiforova M N, Kimura E T, Gandhi M, et al. B-Raf mutations in thyroid tumors are restricted to papillary carcinomas and anaplastic or poorly differentiated carcinomas arising from papillary carcinomas. J Clin Endocrinol Metab. 2003, 88: 5399-5404.

41. Yuen ST, Davies HM, Chan T L, et al. Similarity of the phenotypic patterns associated with B-Raf and KRas mutations in colorectal neoplasia. Cancer Res. 2002, 62: 6451-6455.

42. Brose M S, Volpe P, Feldman M, et al. B-Raf and Ras mutations in human lung cancer and melanoma. Cancer Res. 2002, 62: 6997-7000.

43. Ikenoue T, Hikiba Y, Kanai F, et al. Diffrent effects of point mutations within the B-Raf glycine-rich loop in colorectal tumors on mitogen-activated protein/extracellular signal-regulated kinase kinase/extracellular signal-regulated kinase and nuclear factorκB pathway and cellular transformation. Cancer Res. 2004, 64: 3428-3435.

44. M. K. Borysova, Y. Cui, M. Snyder, and T. M. Guadagno\*. Knockdown of B-Raf

Impairs spindle formation and the mitotic checkpoint in human somatic cells. Cell Cycle. 2008, 7(18), 2894-2901.

45. Cui Y, Borysova MK, Johnson JO, Guadagno TM. Oncogenic B-RafV600EInduces Spindle Abnormalities, Supernumerary Centrosomes, an Aneuploidy in Human Melanocytic Cells. Cancer Research. 2010, 70(2), 675-684.

46. Jing Liu, Xiaolong Cheng, Yanyan Zhang, Shujing Li, Heyang Cui, Ling Zhang, Ruyi Shi, Zhiping Zhao, Chanting He, Chuangui Wang, Haoliang Zhao, Ce Zhang, Harold A. Fisk, Thomas M. Guadagno, Yongping Cui\*. Phosphorylation of Mps1 by BRAFV600E Prevents Mps1 Degradation and Contributes to Chromosome Instability in Melanoma. Oncogene. 2013, 7;32(6):713-23.

47. Fisk HA, Mattison CP, Winey M. Human Mps1 protein kinase is required for centrosome duplication and normal mitotic progressi on. PNAS. 2003, 100: 14875-80.

48. Fisk HA, Winey M. Spindle regulation: Mps1 files into new areas. Curr Biol. 2004, 14: R1058-60.

49. Hoyt MA. Cell biology. Extinguishing a cell cycle checkpoint. Science. 2006, 313: 624-625.

50. Palframan WJ, Meehi JB, Jaspersen SL, Winey M, Murray AW. Anaphase inactivation of the spindle checkpoint. Science. 2006, 313:680-684.

[51. Woo J](http://www.ncbi.nlm.nih.gov/pubmed?term=Woo%20J%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23504768), [Palmisiano N](http://www.ncbi.nlm.nih.gov/pubmed?term=Palmisiano%20N%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23504768), [Tester W](http://www.ncbi.nlm.nih.gov/pubmed?term=Tester%20W%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23504768), [Leighton JC Jr](http://www.ncbi.nlm.nih.gov/pubmed?term=Leighton%20JC%20Jr%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23504768). Controversies in antiepidermal growth factor receptor therapy in metastatic colorectal cancer. [Cancer.](http://www.ncbi.nlm.nih.gov/pubmed/23504768) 2013 Mar 15. doi: 10.1002

[52. Xynos ID](http://www.ncbi.nlm.nih.gov/pubmed?term=Xynos%20ID%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23445638), [Karadima ML](http://www.ncbi.nlm.nih.gov/pubmed?term=Karadima%20ML%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23445638), [Voutsas IF](http://www.ncbi.nlm.nih.gov/pubmed?term=Voutsas%20IF%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23445638), [Amptoulach S](http://www.ncbi.nlm.nih.gov/pubmed?term=Amptoulach%20S%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23445638), [Skopelitis E](http://www.ncbi.nlm.nih.gov/pubmed?term=Skopelitis%20E%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23445638), [Kosmas C](http://www.ncbi.nlm.nih.gov/pubmed?term=Kosmas%20C%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23445638), [Gritzapis AD](http://www.ncbi.nlm.nih.gov/pubmed?term=Gritzapis%20AD%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23445638), [Tsavaris N](http://www.ncbi.nlm.nih.gov/pubmed?term=Tsavaris%20N%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23445638). Chemotherapy±Cetuximab Modulates Peripheral Immune Responses in Metastatic Colorectal Cancer. [Oncology.](http://www.ncbi.nlm.nih.gov/pubmed/23445638) 2013 Feb 22;84(5):273-283.

[53. Jiang Z](http://www.ncbi.nlm.nih.gov/pubmed?term=Jiang%20Z%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23441167), [Li C](http://www.ncbi.nlm.nih.gov/pubmed?term=Li%20C%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23441167), [Li F](http://www.ncbi.nlm.nih.gov/pubmed?term=Li%20F%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23441167), [Wang X](http://www.ncbi.nlm.nih.gov/pubmed?term=Wang%20X%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23441167)EGFR Gene Copy Number as a Prognostic Marker in Colorectal Cancer Patients Treated with Cetuximab or Panitumumab: A Systematic Review and Meta Analysis. [PLoS One.](http://www.ncbi.nlm.nih.gov/pubmed/23441167) 2013, 8(2): e56205. doi: 10.1371

54. Tebbutt NC, Parry MM, Zannino D, Strickland AH, Van Hazel GA, Pavlakis N, Ganju V, Mellor D, Dobrovic A, Gebski VJ. Docetaxel plus cetuximab as second-line treatment for docetaxel-refractory oesophagogastric cancer: the AGITG ATTAX2 trial. [Br J Cancer.](http://www.ncbi.nlm.nih.gov/pubmed/23412099) 2013, Mar 5;108(4):771-4.

**55** [Heinemann V](http://www.ncbi.nlm.nih.gov/pubmed?term=Heinemann%20V%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23375249), [Douillard JY](http://www.ncbi.nlm.nih.gov/pubmed?term=Douillard%20JY%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23375249), [Ducreux M](http://www.ncbi.nlm.nih.gov/pubmed?term=Ducreux%20M%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23375249), [Peeters M](http://www.ncbi.nlm.nih.gov/pubmed?term=Peeters%20M%5BAuthor%5D&amp;cauthor=true&amp;cauthor_uid=23375249). Targeted therapy in metastatic colorectal cancer -An example of personalised medicine in action. [Cancer Treat Rev.](http://www.ncbi.nlm.nih.gov/pubmed/23375249) 2013, pii: S0305-7372(13) 00006-6

56. M. K. Borysova, Y. Cui, M. Snyder, and T. M. Guadagno\*. Knockdown of B-Raf impairs spindle formation and the mitotic checkpoint in human somatic cells. Cell Cycle. 2008, 7(18), 2894-2901.

57. Cui Y, Borysova MK, Johnson JO, Guadagno TM. Oncogenic B-RafV600EInduces Spindle Abnormalities, Supernumerary Centrosomes, an Aneuploidy in Human Melanocytic Cells. Cancer Research. 2010, 70(2), 675-684.

58. Weeraratna AT. [RAF around the edges--the paradox of BRAF inhibitors.](http://www.ncbi.nlm.nih.gov/pubmed/22256810) N Engl J Med. 2012, 366: 271-273.

59. Su F, Viros A, Milagre C, Trunzer K, Bollag G, Spleiss O, Reis-Filho JS, Kong X,

Koya RC, Flaherty KT, Chapman PB, Kim MJ, Hayward R, Martin M, Yang H, Wang Q, Hilton H, Hang JS, Noe J, Lambros M, Geyer F, Dhomen N, Niculescu-Duvaz I, Zambon A, Niculescu-Duvaz D, Preece N, Robert L, Otte NJ, Mok S, Kee D, Ma Y, Zhang C, Habets G, Burton EA, Wong B, Nguyen H, Kockx M, Andries L, Lestini B, Nolop KB, Lee RJ, Joe AK, Troy JL, Gonzalez R, Hutson TE, Puzanov I, Chmielowski B, Springer CJ, McArthur GA, Sosman JA, Lo RS, Ribas A, Marais R. [RAS mutations in cutaneous squamous-cell carcinomas in patients treated with](http://www.ncbi.nlm.nih.gov/pubmed/22256804) [BRAF inhibitors.](http://www.ncbi.nlm.nih.gov/pubmed/22256804) N Engl J Med. 2012, 366:207-215.

[60. Prahallad A](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Prahallad%20A%22%5BAuthor%5D), [Sun C](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Sun%20C%22%5BAuthor%5D), [Huang S](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Huang%20S%22%5BAuthor%5D), [Di Nicolantonio F](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Di%20Nicolantonio%20F%22%5BAuthor%5D), [Salazar R](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Salazar%20R%22%5BAuthor%5D), [Zecchin D](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Zecchin%20D%22%5BAuthor%5D), [Beijersbergen RL](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Beijersbergen%20RL%22%5BAuthor%5D), [Bardelli A](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Bardelli%20A%22%5BAuthor%5D), [Bernards R](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Bernards%20R%22%5BAuthor%5D). Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. [Nature.](http://www.ncbi.nlm.nih.gov/pubmed/22281684#%23) 2012, 483(7387), 100-3.

[61. Königsberg R](http://www.ncbi.nlm.nih.gov/pubmed?term=%22K%C3%B6nigsberg%20R%22%5BAuthor%5D), [Hulla W](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Hulla%20W%22%5BAuthor%5D), [Klimpfinger M](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Klimpfinger%20M%22%5BAuthor%5D), [Reiner-Concin A](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Reiner-Concin%20A%22%5BAuthor%5D), [Steininger T](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Steininger%20T%22%5BAuthor%5D), [Büchler W](http://www.ncbi.nlm.nih.gov/pubmed?term=%22B%C3%BCchler%20W%22%5BAuthor%5D), [Terkola R](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Terkola%20R%22%5BAuthor%5D), [Dittrich C](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Dittrich%20C%22%5BAuthor%5D). Clinical and Economic Aspects of KRAS Mutational Status as Predictor for Epidermal Growth Factor Receptor Inhibitor Therapy in Metastatic Colorectal Cancer Patients. [Oncology.](http://www.ncbi.nlm.nih.gov/pubmed/22248908#%23) 2011, 81(5-6):359-64.

62. Weeraratna AT. [RAF around the edges--the paradox of BRAF inhibitors.](http://www.ncbi.nlm.nih.gov/pubmed/22256810) N Engl J Med. 2012, 366(3):271-3.

[63. Johannessen CM](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Johannessen%20CM%22%5BAuthor%5D), [Boehm JS](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Boehm%20JS%22%5BAuthor%5D), [Kim SY](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Kim%20SY%22%5BAuthor%5D), [Thomas SR](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Thomas%20SR%22%5BAuthor%5D), [Wardwell L](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Wardwell%20L%22%5BAuthor%5D), [Johnson LA](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Johnson%20LA%22%5BAuthor%5D), [Emery CM](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Emery%20CM%22%5BAuthor%5D), [Stransky N](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Stransky%20N%22%5BAuthor%5D), [Cogdill AP](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Cogdill%20AP%22%5BAuthor%5D), [Barretina J](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Barretina%20J%22%5BAuthor%5D), [Caponigro G](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Caponigro%20G%22%5BAuthor%5D), [Hieronymus H](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Hieronymus%20H%22%5BAuthor%5D), [Murray RR](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Murray%20RR%22%5BAuthor%5D), [Salehi-Ashtiani K](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Salehi-Ashtiani%20K%22%5BAuthor%5D), [Hill DE](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Hill%20DE%22%5BAuthor%5D), [Vidal M](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Vidal%20M%22%5BAuthor%5D), [Zhao JJ](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Zhao%20JJ%22%5BAuthor%5D), [Yang X](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Yang%20X%22%5BAuthor%5D), [Alkan O](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Alkan%20O%22%5BAuthor%5D), [Kim S](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Kim%20S%22%5BAuthor%5D), [Harris JL](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Harris%20JL%22%5BAuthor%5D), [Wilson CJ](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Wilson%20CJ%22%5BAuthor%5D), [Myer VE](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Myer%20VE%22%5BAuthor%5D), [Finan PM](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Finan%20PM%22%5BAuthor%5D), [Root DE](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Root%20DE%22%5BAuthor%5D), [Roberts TM](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Roberts%20TM%22%5BAuthor%5D), [Golub T](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Golub%20T%22%5BAuthor%5D), [Flaherty KT](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Flaherty%20KT%22%5BAuthor%5D), [Dummer R](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Dummer%20R%22%5BAuthor%5D), [Weber BL](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Weber%20BL%22%5BAuthor%5D), [Sellers WR](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Sellers%20WR%22%5BAuthor%5D), [Schlegel R](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Schlegel%20R%22%5BAuthor%5D), [Wargo JA](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Wargo%20JA%22%5BAuthor%5D), [Hahn WC](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Hahn%20WC%22%5BAuthor%5D), [Garraway LA](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Garraway%20LA%22%5BAuthor%5D). COT drives resistance to RAF inhibition through MAP kinase pathway

Reactivation. Nature. 2010, 468(7326)**,** 968–972.

[64. Villanueva J](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Villanueva%20J%22%5BAuthor%5D), [Vultur A](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Vultur%20A%22%5BAuthor%5D), [Lee JT](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Lee%20JT%22%5BAuthor%5D), [Somasundaram R](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Somasundaram%20R%22%5BAuthor%5D), [Fukunaga-Kalabis M](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Fukunaga-Kalabis%20M%22%5BAuthor%5D), [Cipolla AK](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Cipolla%20AK%22%5BAuthor%5D), [Wubbenhorst B](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Wubbenhorst%20B%22%5BAuthor%5D), [Xu X](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Xu%20X%22%5BAuthor%5D), [Gimotty PA](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Gimotty%20PA%22%5BAuthor%5D), [Kee D](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Kee%20D%22%5BAuthor%5D), [Santiago-Walker AE](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Santiago-Walker%20AE%22%5BAuthor%5D), [Letrero R](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Letrero%20R%22%5BAuthor%5D), [D'Andrea K](http://www.ncbi.nlm.nih.gov/pubmed?term=%22D%27Andrea%20K%22%5BAuthor%5D), [Pushparajan A](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Pushparajan%20A%22%5BAuthor%5D), [Hayden JE](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Hayden%20JE%22%5BAuthor%5D), [Brown KD](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Brown%20KD%22%5BAuthor%5D), [Laquerre S](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Laquerre%20S%22%5BAuthor%5D), [McArthur GA](http://www.ncbi.nlm.nih.gov/pubmed?term=%22McArthur%20GA%22%5BAuthor%5D), [Sosman JA](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Sosman%20JA%22%5BAuthor%5D), [Nathanson KL](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Nathanson%20KL%22%5BAuthor%5D), [Herlyn M](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Herlyn%20M%22%5BAuthor%5D). [Acquired resistance to BRAF inhibitors mediated by a RAF kinase switch in melanoma can be overcome by cotargeting MEK and IGF-1R/PI3K.](http://www.ncbi.nlm.nih.gov/pubmed/21156289) Cancer Cell. 2010, 18(6), 683-695.

# 个人简历

**姓名**：刘静**性别**：女

**出生日期**：1973.1**出生地**：ft西太原

**最后学历**：研究生**政治面貌**：群众

**学位**：硕士**毕业院校**：ft西医科大学

**学习工作经历：**

|  |  |  |
| --- | --- | --- |
|  1991 年-1996 年 | ft西医科大学 | 临床医学专业 |
|  2000 年-2003 年 | ft西医科大学 | 外科学专业 |
|  2010 年-至今 | ft西医科大学 | 外科学专业 |

1996年大学毕业分配至ft西医科大学第一医院工作至今

**攻读博士学位期间承担及参与的课题：**

1. Mps1基因在携带B-RAFV600E突变型恶性肿瘤靶向治疗中的作用2012年国家自然科学基金青年基金项目（编号: 81201956）第一负责人

2. 癌基因B-RAFV600E导致两极纺锤体结构及定位异常的分子机制2012年国家自然科学基金面上项目（编号: 81272189）第二参与人

3. TCA短串联重复序列多肽对ECRG2基因功能的影响及机制研究2012年ft西医科大学校创新（编号: 01201109）第一负责人

4. 癌基因B-RafV600E对纺锤体检测点功能及中心体复制的影响2011年ft西省研究生创新项目（编号: 2010089）第一负责人已结题

**攻读博士学位期间公开发表的论文：**

1. Jing Liu, Xiaolong Cheng, Yanyan Zhang, Shujing Li, Heyang Cui, Ling Zhang, Ruyi Shi, Zhiping Zhao, Chanting He, Chuangui Wang, Haoliang Zhao, Ce Zhang, Harold A. Fisk, Thomas M. Guadagno, Yongping Cui\*. Phosphorylation of Mps1 by BRAFV600E Prevents Mps1 Degradation and Contributes to Chromosome Instability in Melanoma. Oncogene. 2013,

7;32(6):713-23. ***IF: 7.414***

2. 景永茂，**刘静\***，李曙晶，史卫俊，成晓龙. COX－2启动子区遗传变异与胃癌发病风险的关系. 中国优生与遗传杂志.2012,20(5)：24-25,30.

3. **刘静**，李曙晶，史卫俊，成晓龙. 凋亡通路Fas /Fasl遗传变异与胃癌发病风险的关系.中国优生与遗传杂志.2012, 20(8)：28-30.

4. 景永茂，**刘静\***. 甲状腺微小癌临床诊治体会（附36例临床分析）. 中国药物与临床.2011,11(12)：1449-1450.

5. **刘静**，尚凡晶，刘建生，罗飞. 人乳头状瘤病毒感染与乳腺癌的关系及与P16蛋白表达的相关性研究。中国药物与临床.2011,11(2)：147-150.

致 **谢**

时光如梭，三年的博士学习生涯转眼即逝。蓦然回首，往事历历在目。此时此刻，心中感触良多，而更多的还是一股感激之情。

首先衷心感谢我敬爱的导师崔永萍教授。本论文是在崔永萍教授的悉心指导下完成的。导师渊博的专业知识，严谨的治学态度，精益求精的工作作风，诲人不倦的高尚师德，对我影响深远。本论文从选题设计到完成，每一步都是在导师的指导下完成的，倾注了她大量的心血！在此，谨向导师表示崇高的敬意和衷心的感谢！

感谢我的第二导师赵浩亮教授，作为我省著名的普通外科专家，您崇高的人格魅力、渊博的学识以及忘我的工作精神，是我人生道路的一盏明灯，是我永远学习的典范！在此，向您表示崇高的敬意和衷心的感谢！

感谢ft西医科大学成晓龙老师在课题研究过程以及学习和生活等方面中给予的无私的帮助和大力支持。

感谢国家自然基金面上项目（编号: 30872932; 30971518）ft西省卫生厅科技攻关计划项目（编号：200935）对本课题的资助。

感谢普外科全体同仁、胸外科及病理科领导同事在我攻读学位的三年里对于临床标本方面给予的大力支持和帮助。

感谢同门师弟、师妹们，你们与我一起同甘共苦，认真的学习态度，不计较个人得失的品格让我感动，祝愿你们今后乘风破浪，前途无量！

感谢三年来一直默默支持我的家人和朋友们！

滴水之恩，当涌泉相报。我唯有以巨大的热情投入到工作和学习中去，来报答所有关心过我，帮助过我的人！