

CRISPR/Cas9 mediated BRAF mutation and its influencing factors in papillary thyroid carcinoma cells

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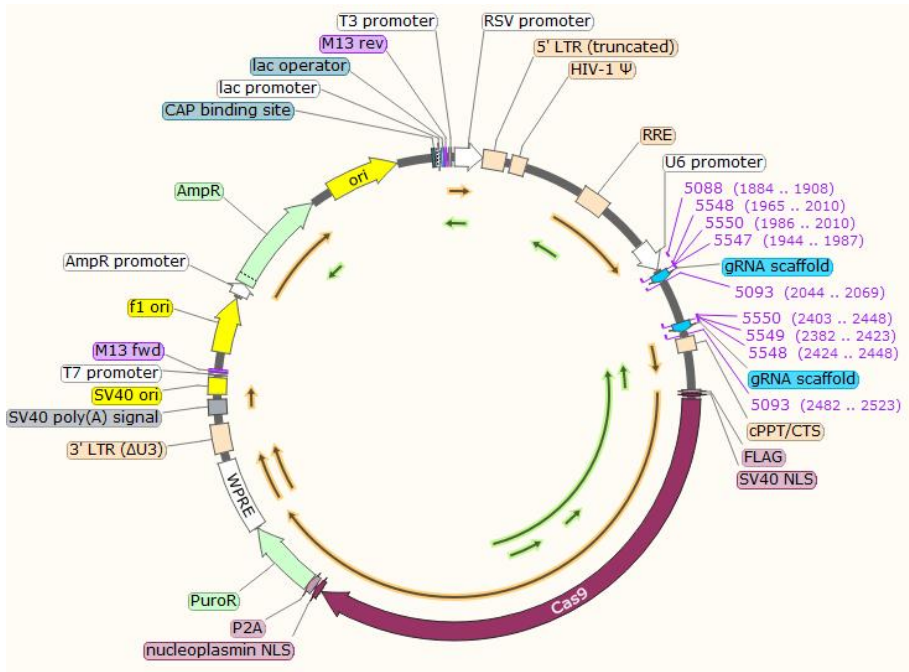
Introduction:

BRAF mutation is the most common and important mutation in thyroid carcinoma. Many studies have focused on the biological behavior of thyroid carcinoma after BRAF mutation, but how this mutation happened was still poorly defined. Our study aims to observe whether the BRAF mutation could be induced by CRISPR/Cas9, also to find the factors which might affect the BRAF

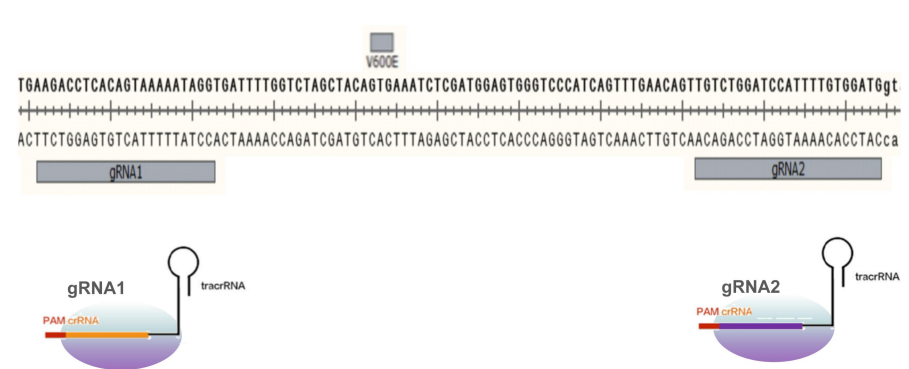
Primary organ site	Frequency of point mutation*	
	KRAS	BRAF
Thyroid	1.8% (141/7,717)	41.5% (19,297/46,463)
Skin	2.3% (86/3,729)	41.4% (8,134/19,667)
Large intestine	34.5% (18,551/53,826)	12.5% (9,253/74,074)
Eye	1.6% (4/258)	10.1% (84/828)
Bone	1.7% (11/643)	9.6% (53/552)
Hematopoietic and lymphoid	4.5% (532/11,956)	9.1% (786/8,636)
Pituitary	0% (0/315)	8.7% (20/230)
Central nervous system	0.9% (28/3,264)	7.0% (392/5,598)
Ovary	11.7% (660/5,653)	6.2% (270/4,386)
Biliary tract	23.3% (631/2,707)	5.8% (50/865)

Genetic mutations in different cancers

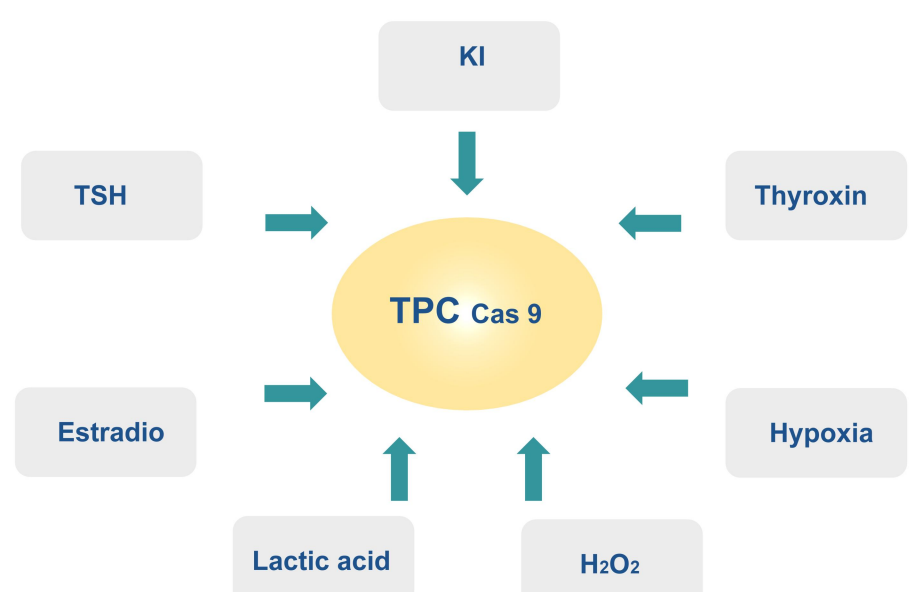
Materials and method:



BRAF 15th exon:



Construction of CRISPR/Cas9 plasmid



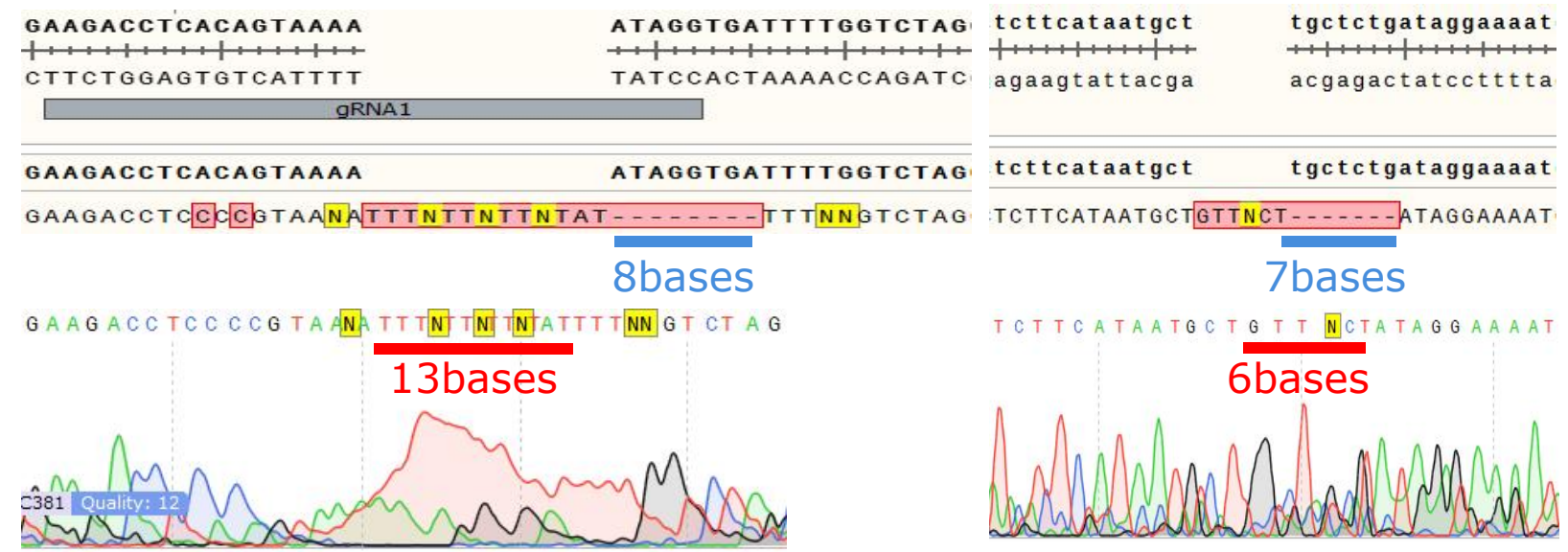
Factors influencing BRAF mutation

Results:

The BRAF mutations including mismatch, insertion and deletion could be observed in the targeting region of the 15th exon. The mutation rates was 16.7%. Besides, it also could be up regulated by TSH, Estradio, Lactic acid and hypoxia with the mutation rates of 41.7%, 53.8%, 21.7%, 53.8% respectively, but only the differences in Estradio and hypoxia groups have statistic significances. ($\chi^2=5.58$, $p=0.018$; $\chi^2=6.55$, $p=0.011$, respectively)



BRAF mutation - mismatch



BRAF mutation - insertion and deletion



BRAF mutation - mismatch

	Mutation	Normal	Mutation ratio(%)	χ^2	p value
TPC Cas9	4	20	16.7		
▲ TSH	10	14	41.7	3.63	0.057
KI	4	19	17.4	0.004	0.947
Thyroxin	2	20	9.1	0.581	0.446
▲ Estradiol	7	6	53.8	5.58	0.018*
▲ Lactic acid	5	18	21.7	0.195	0.659
▲ Hypoxia	14	10	58.3	6.545	0.011*

The mutation rates in different conditions

Conclusion:

BRAF mutation could be induced by CRISPR/Cas9 in papillary thyroid carcinoma cells, it also could be up regulated by hypoxia and Estradio.