Εύρηκα

研究 CTEN 在大腸癌細胞中的影響

報告人: 江祐辰
指導老師: 魏宏仁
指導教授: 廖憶純

背景

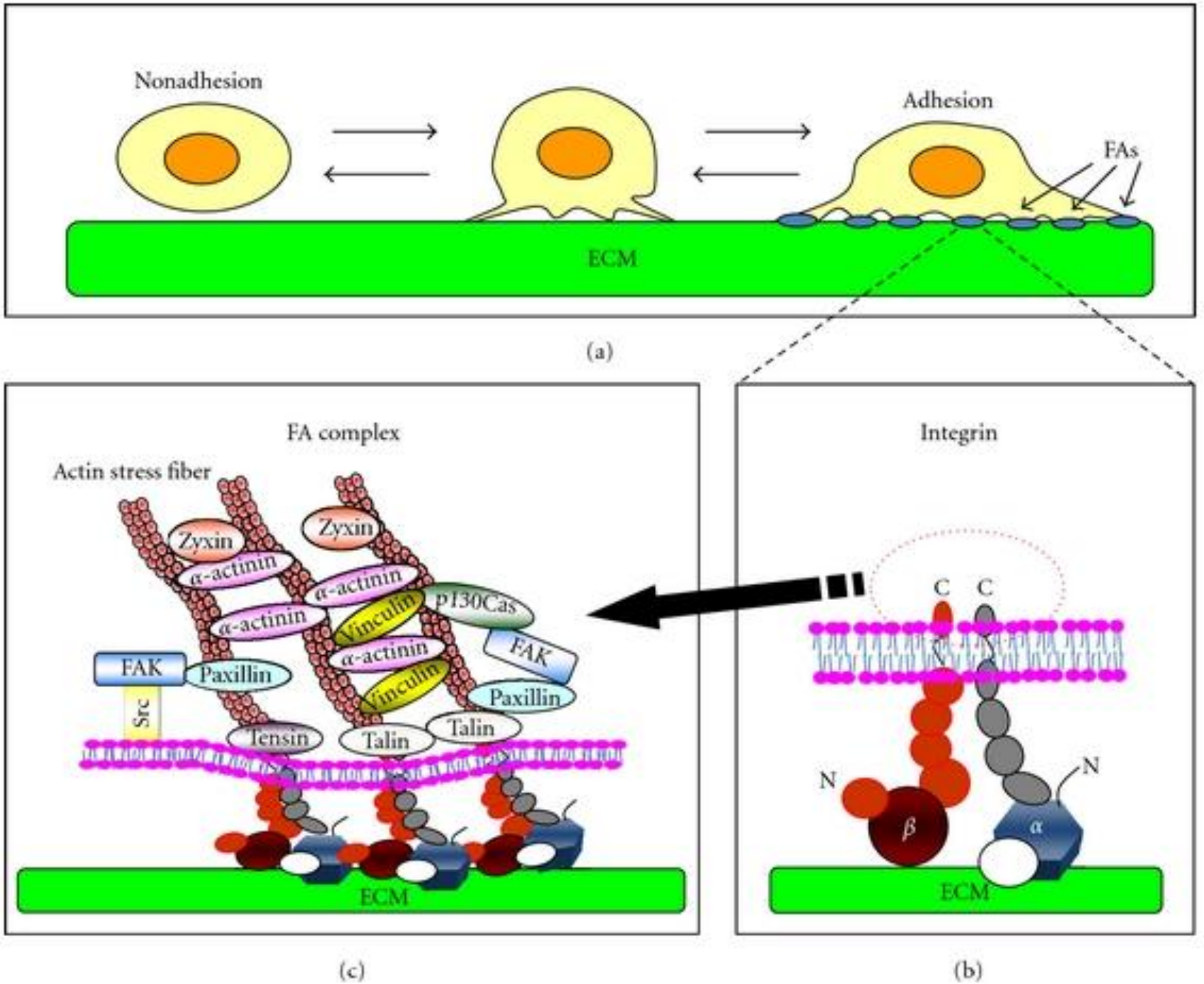
研究動機

- 台灣是個美食天堂
- 外食頻繁
- 大腸癌是台灣罹患率最高的癌症之一

CT

- C
集中
胞附
要角

- CT
過程



質，
於細
寅重
生。

遷移的

文獻

- 正常情況下 CTEN 表現量低(集中附著點)
- 大量 (表現量高)的 CTEN 也在大腸癌細胞的細胞核中被發現，這是正常細胞中沒有被觀察到的現象
- CTEN 在大腸癌細胞核中可與 beta-catenin 結合並促進腫瘤發生。

目標

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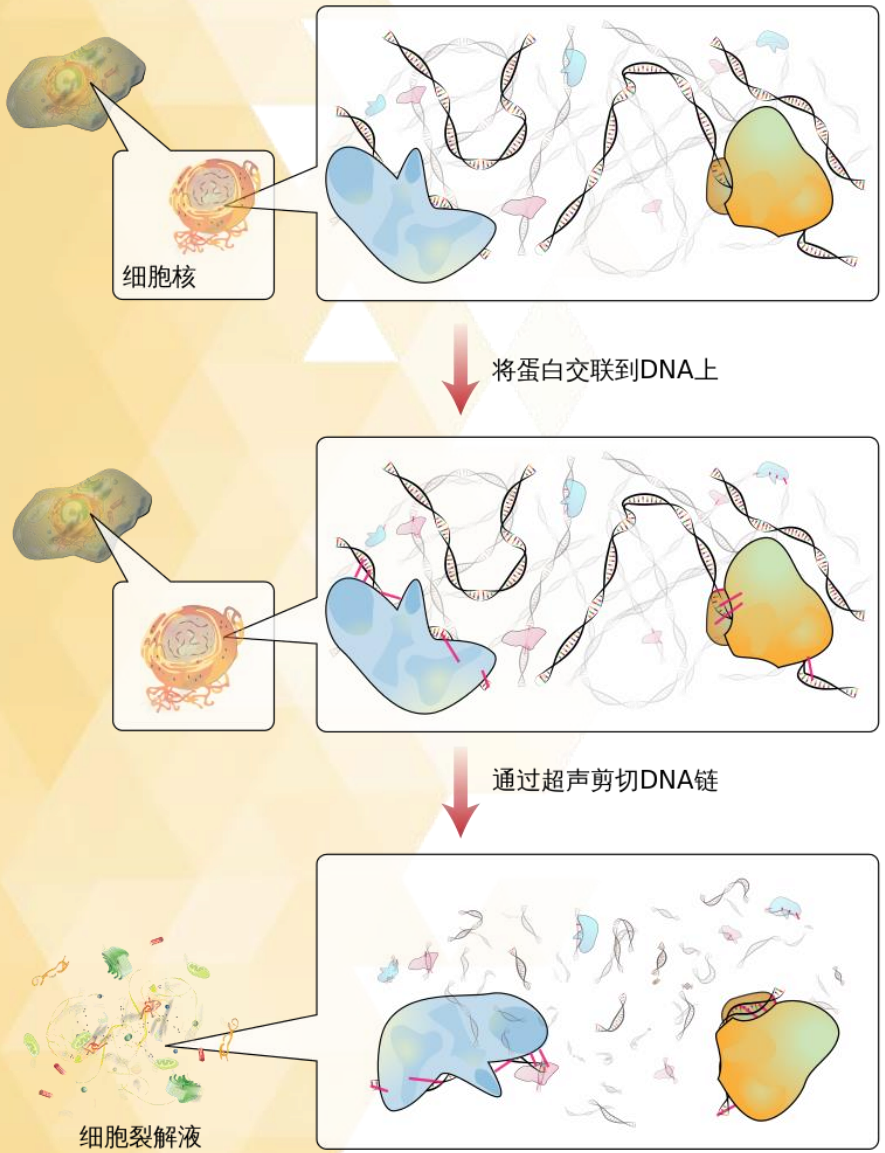
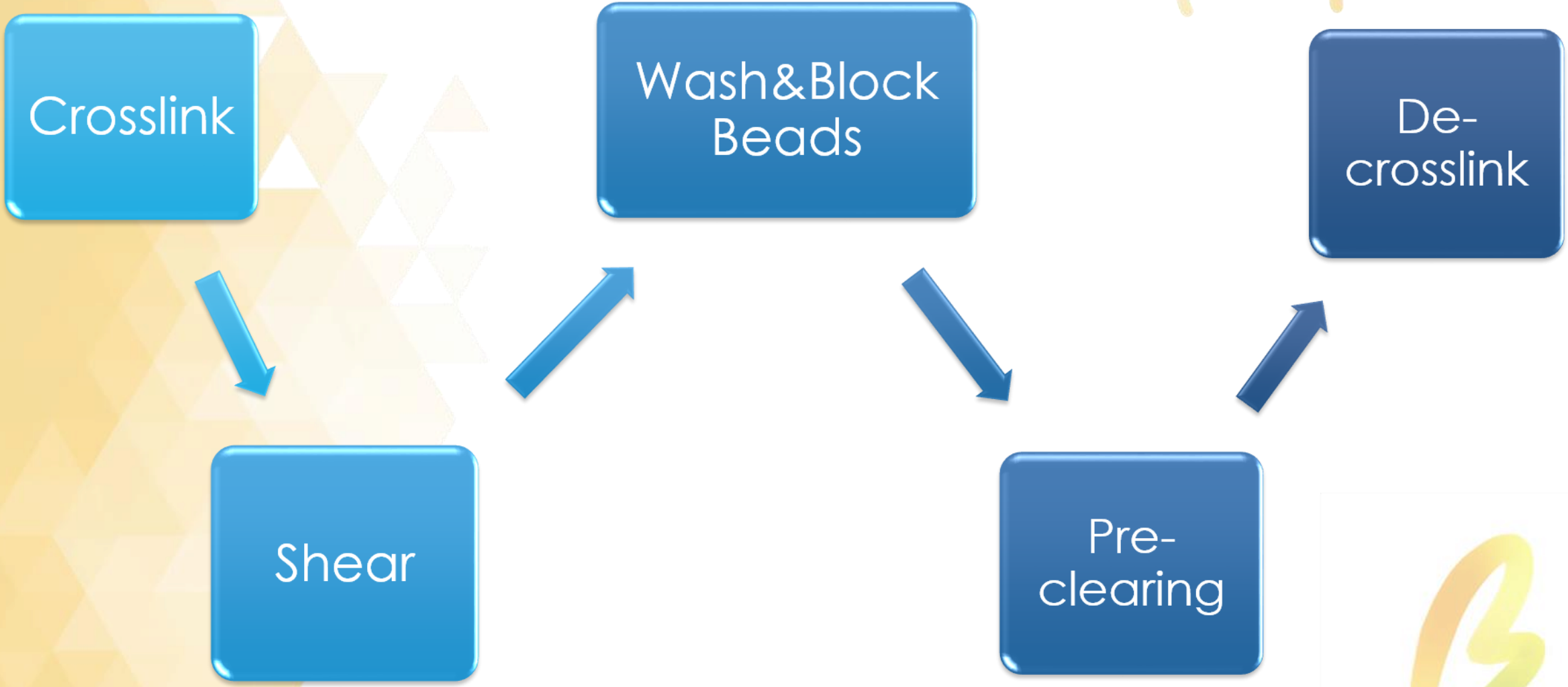
- 瞭解位於細胞核的 CTEN 在腫瘤發生過程中所扮演的角色，進而闡明 CTEN 促進腫瘤發生的分子機轉，預期可進而提供發展癌症治療的新標的。

方法

染色質免疫沉澱

- Chromatin Immunoprecipitation
- 簡稱ChIP
- 功能：確定特定蛋白是否結合特定基因組區域


细胞核
将蛋白交联到DNA上
通过超声剪切DNA链
细胞裂解液
加入附上抗体的珠以免疫沉淀靶蛋白
沉淀
解除蛋白交联；纯化DNA
测序
标记到基因组上
ATGCCTGGACCGTG


Crosslink
Shear
Wash&Block Beads
Pre-clearing
De-crosslink

步驟(Crosslink)

1
- PBS潤洗細胞
- 加入 DMEM & 甲醛 開始 Crosslink

2
- 加入 甘胺酸 停止 Crosslink
- PBS潤洗細胞

3
- 加入PBS將細胞刮入離心管，離心後丟掉上清液[+C]
- 加入PBS，分裝到微量離心管+C

步驟(Shearing)

1
- 以 Buffer A & NP40 [P.I.] 懸浮細胞(震盪)+C
- 以 MNase rxn buffer 懸浮。加入 Mnase，37℃乾浴

2
- 加入 MNase stop buffer+C
- 以 RIPA Lysis Buffer 懸浮

3
- 超音波剪切
- 離心後收集上清液* (取約1/4為Input)

步驟(wash&block beads)

1
- 震盪agarose beads，分裝至微量離心管
- 加dilution buffer，離心後置冰上一分鐘，丟掉上清液x2

2
- 加入BSA(小牛血清白蛋白)&dilution buffer
- Rotate for 1hr (4℃)

3
- 加dilution buffer，離心後置冰上一分鐘，丟掉上清液
- 加dilution buffer 沖掉多餘的bead& 重新懸浮

步驟(Pre-clearing)

1
- 加 dilution buffer in [P.I.]& BSA-blocked beads
- 4℃水浴 1 hr 後，離心，收集上清液到新的微量離心管

2
- 加入抗體， 4℃水浴 16 hr
- 加入 blocked beads，4℃ rotate 2 hr

3
- 離心後置冰上一分鐘
- 以 low-salt wash buffer 沖洗(緩慢pipet)+C

步驟(Pre-clearing)

4
- 將混合液裝至新的微量離心管+C
- 以high salt wash buffer 沖洗+C

5
- 以 LiCl wash buffer 沖洗+C
- 以 TE Buffer 沖洗，裝至新微量離心管+C

6
- 加 ChIP elution buffer，65℃水浴搖晃半小時
- 離心，將上清液裝到新的微量離心管

步驟(De-crosslinking)

- 融化Input，加入elution buffer
- 加 5M NaCl到每個 sample，65℃水浴反應

- 加 Tris-HCl(ph6.8) & EDTA
- 加 Protein K

- 65℃水浴
- 純化DNA

步驟(Crosslink)

- PBS rinse cell
- Refill 9ml DMEM+270μl 37% formaldehyde, shake 10min at regular temperature(@RT)
- Add 1ml 1.25M Glycine, shake 5min @RT
- Wash with icy PBS× 2
- Add 3ml icy PBS to each plate and scrape it into 15ml falcon tube
- Centrifuge (1000g, 5min @4℃), discard supernatant
- Suspended with PBS, transfer 1ml to each eppendorf

步驟(Shearing)

- Centrifuge (2000g, 5min @4℃), discard supernatant
- Suspended with [100µl BufferA+7µl NP40] ([P.I.])
- Vortex, rest on ice for 2min, vortex
- Centrifuge (2000g, 5min @4℃), discard supernatant
- Suspended with 360µl MNase rxn buffer
- Add 1ml MNase, dry bath @37℃, flip every 5min
- Add 80µl MNase stop buffer

步驟(Shearing)

- Centrifuge (9000g, 5min @4℃), discard supernatant
- Suspended with 400μl RIPA lysis Buffer×[P.I.]
- Sonicate:[10W, on 30sec ; off 30sec] ×4
- Centrifuge (8000rpm, 10min @4℃)
- Collect supernatant*, take aside approximately ¼ as “Input”
- Store @80℃ or move on

步驟(Wash&Block Beads)

(do it 2hr beforehand)

- Vortex agarose beads until fully suspended
- Transfer 25μl beads to each eppendorf, dilute to 1ml by dilution buffer
- Centrifuge (2000g, 1min @4℃), let beads settle on ice for 1min, discard supernatant
- Add 1ml dilution buffer, invert tubes 5 times
- Centrifuge (2000g, 1min @4℃), let beads settle on ice for 1min, discard supernatant

步驟(Wash&Block Beads)

- Add 100µl 10mg/ml BSA & 900µl dilution buffer
- Rotate for 1hr @4℃
- Centrifuge (2000g, 1min @4℃), let beads settle on ice for 1min, discard supernatant
- Wash beads once with 1ml dilution buffer
- Suspended with 25µl dilution buffer

步驟(Pre-clearing)

- Add 900µl dilution buffer(P.I.)
- Add 25µl BSA-blocked beads
- Incubate @4℃ F-wheel for 1hr
- Centrifuge (7500rpm, 3min @4℃), collect supernatant to other eppendorf
- Add antibodies to each eppendorf, incubate 16hr @4℃
- Add 50µl blocked beads, rotate for 2hr @4℃
- Centrifuge (2000g, 1min @4℃), let beads settle on ice for 1min

步驟

- Wash with high salt wash buffer, centrifuge, discard supernatant
- Wash with 1ml LiCl wash buffer, centrifuge, discard supernatant
- Wash with 1ml TE wash buffer, transfer mixure to the eppendorf, centrifuge, discard supernatant
- Add 210µl CHIP elution buffer, incubate shaking for 30min @65℃, centrifuge(11000rpm,3min @4℃)
- Transfer 200µl of supernatant to the new eppendorf

步驟(De-crosslinking)

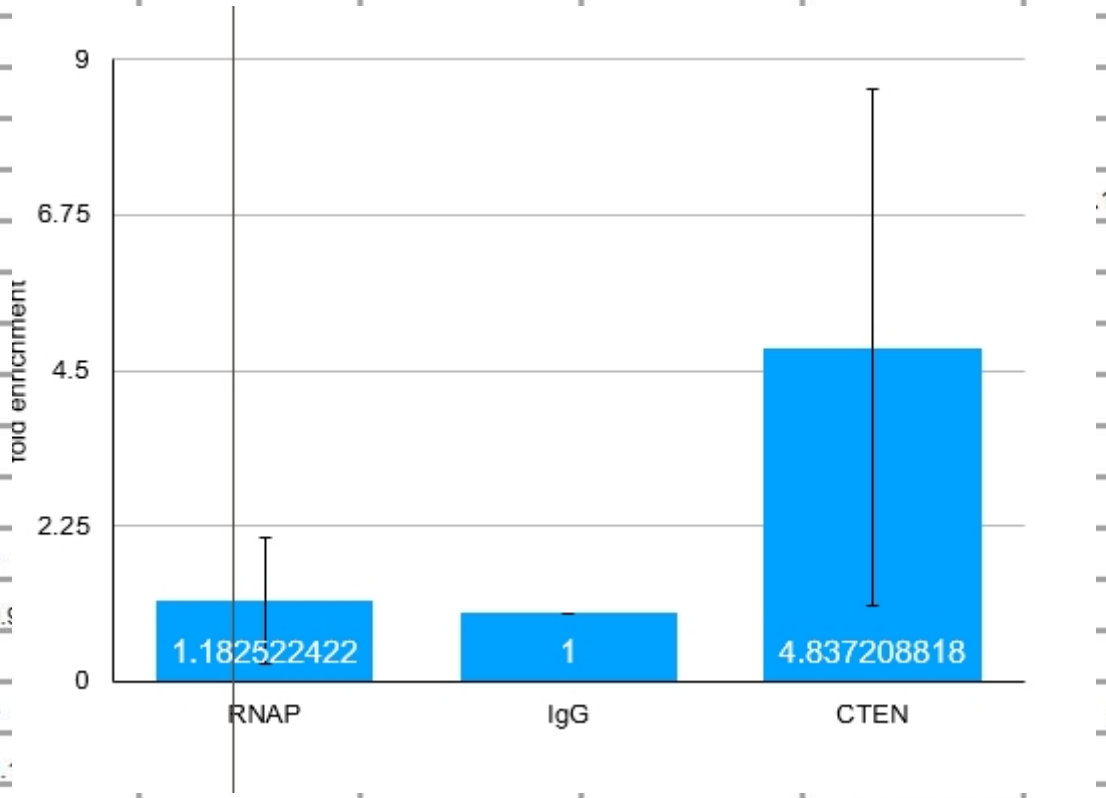
- Thaw input and add elution buffer to 200µl
- Add 8µl of 5M NaCl to each sample, incubate, shake at 65℃ O/N
- Add 20µl 1M Tris-HCl (pH6.8) & 20µl 0.25M EDTA
- Add 2µl Proteinase K($20mg/ml$)
- Incubate and shake for 1hr @60℃

- Purify DNA (kit: GP1002)

跑C

- 以 D

	DHX32-1	DHX32-2	DHX32-3	Average	Average*	Delta-Ct	fold enrichment
Input (5%)	21.67	21.51	20.91	21.36333333	17.04140524		
RNAP	28.24	28.28	26.1	27.54	27.54	2.256666667	0.209254903
IgG	25.43	25.27	25.15	25.28333333	25.28333333	0	1
CTEN	23.64	25.11	24.29	24.34666667	24.34666667	-0.936666667	1.914100614
Input (5%)							
RNAP						-1.02	2.02791896
IgG						0	1
CTEN						.176666667	9.042155114
Input (100%)							
RNAP						-0.39	1.310393404
IgG						0	1
CTEN						-1.83	3.555370725
STDEV	RNAP						
	0.9						
Average	RNAP					2.02791896	1.310393404
	1.1					1	1
					1.914100614	9.042155114	3.555370725
TTEST	RNAP vs IgG		CTEN vs IgG				
	0.747395234		0.149606743				



目前結論

跑基因定序後發現
Cten 會和與細胞週期、移動有關的基因接合。

未來展望

- 釐清運送 CTEN 至細胞核的機制
- 細胞核 CTEN 與促進腫瘤發生的關連性。

參考資料

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- https://ndltd.ncl.edu.tw/cgi-bin/gs32/gsweb.cgi/login?o=dnclcdr&s=id=%22102NTU05106070%22.&searchmode=basic
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- https://www.viogene.com/products/39

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Thank You