中央研究院
基因體研究中心
Genomics Research Center
ACADEMIA SINICA

揭示異常糖代謝和微環境改變在啟動胰臟癌發展的意義

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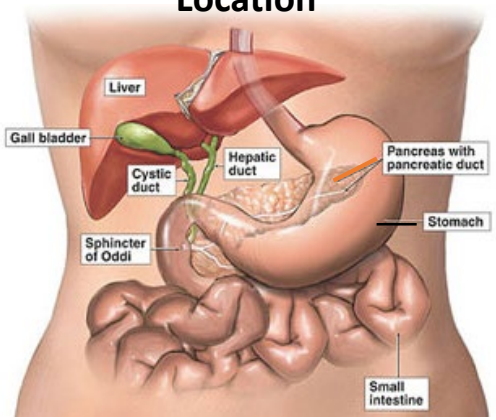
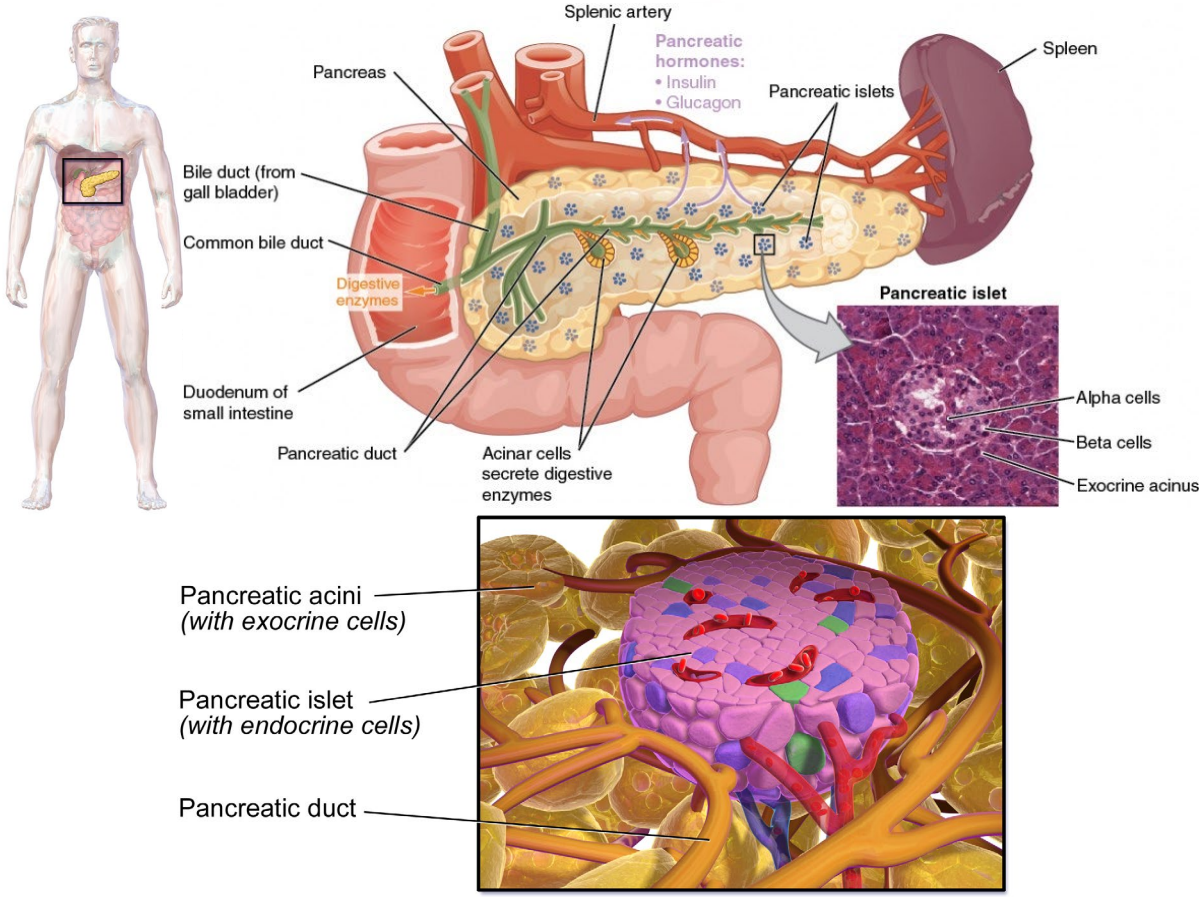
所屬單位: 中央研究院基因體中心
日期：02/24/2024

胰臟在身體中所在的位置和其功能

Location



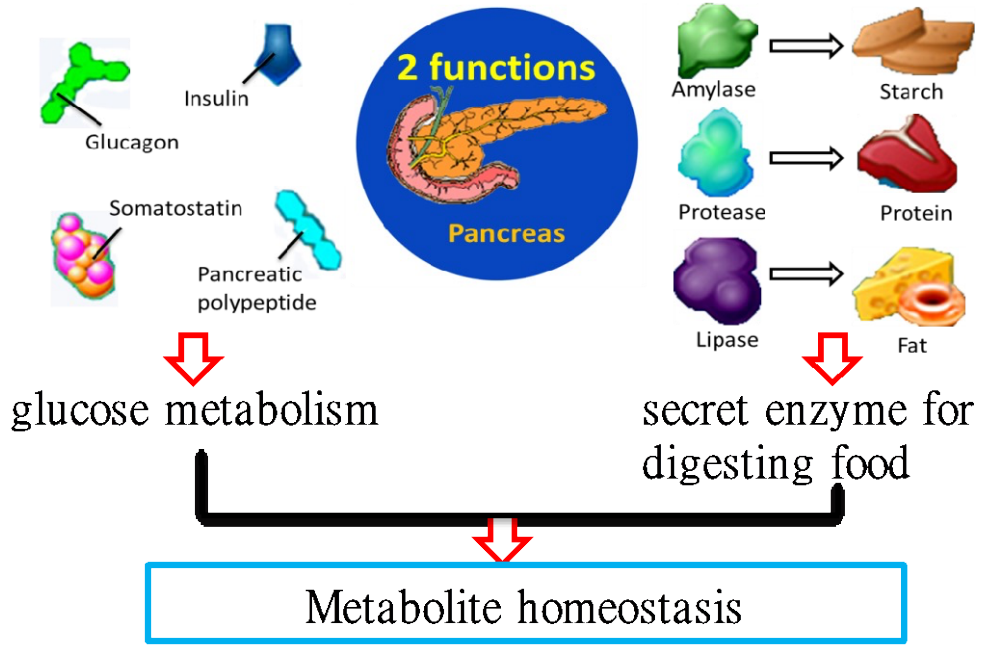
The *pancreas* is *located deep* inside the upper abdomen behind the stomach



Two major functions of pancreas

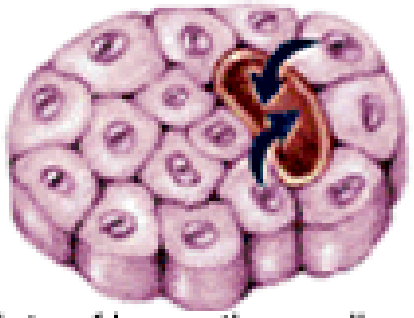
Endocrine
The pancreas produces chemical (hormones) that regulate blood sugar

Exocrine
The pancreas produces enzyme that help digest our food



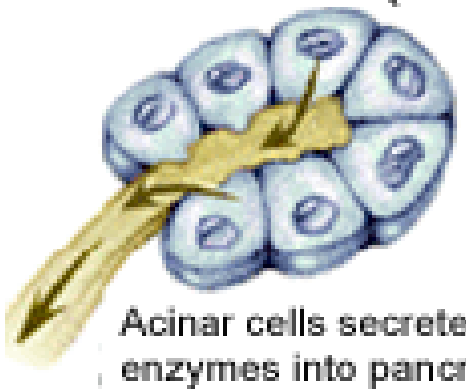
胰臟癌
兩個主要類型

Endocrine (In)



Islets of Langethan cells secrete hormons into blood vessels

Exocrine (out)



Acinar cells secrete pancreatic enzymes into pancreatic duct

- **Minor type is pancreatic neuroendocrine tumor (PNET) (~5 %)**

from endocrine cells → **Islet cells**

- **Major type is pancreatic ductal adenocarcinoma (PDAC) (over 90 %)**

from exocrine cells → **ductal cells**

from exocrine cells → **acinar to ductal –like cells**

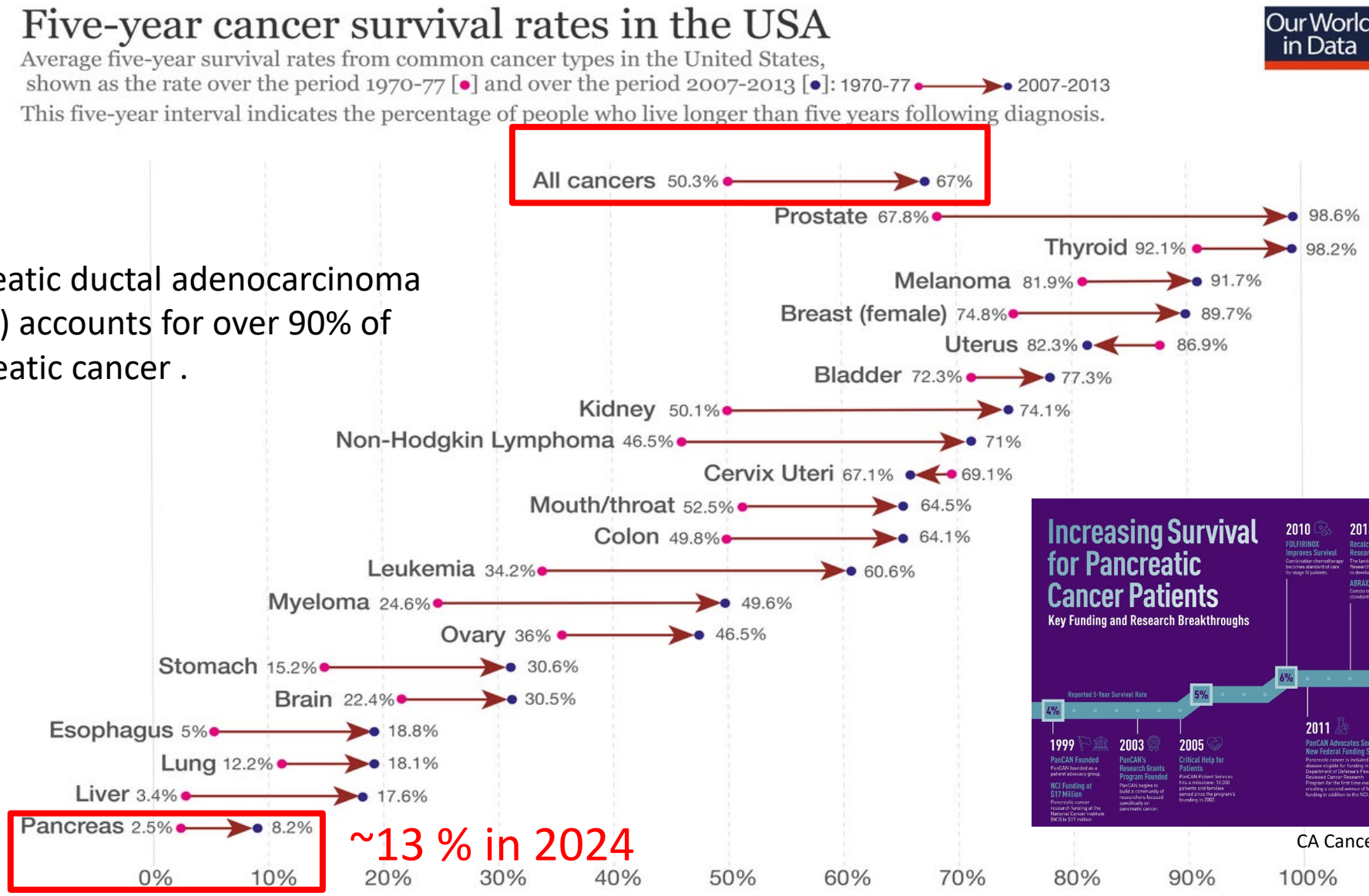
5 year survival rate about 20~40 %

胰臟神經內分泌腫瘤

胰腺癌

胰腺癌是存活率最低的癌症，被稱為“癌中之王”

Pancreatic ductal adenocarcinoma (PDAC) accounts for over 90% of pancreatic cancer .

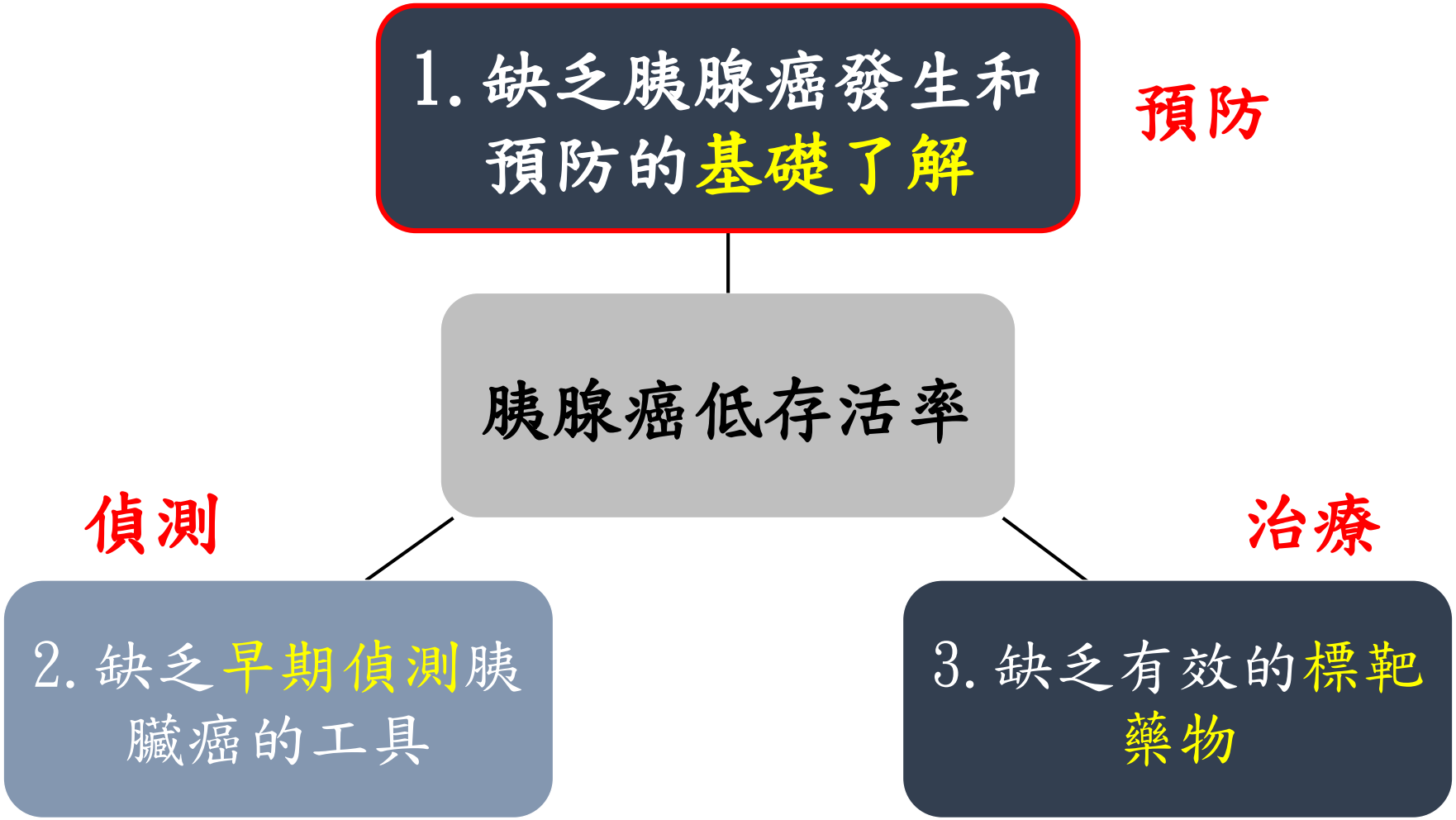


~13 % in 2024

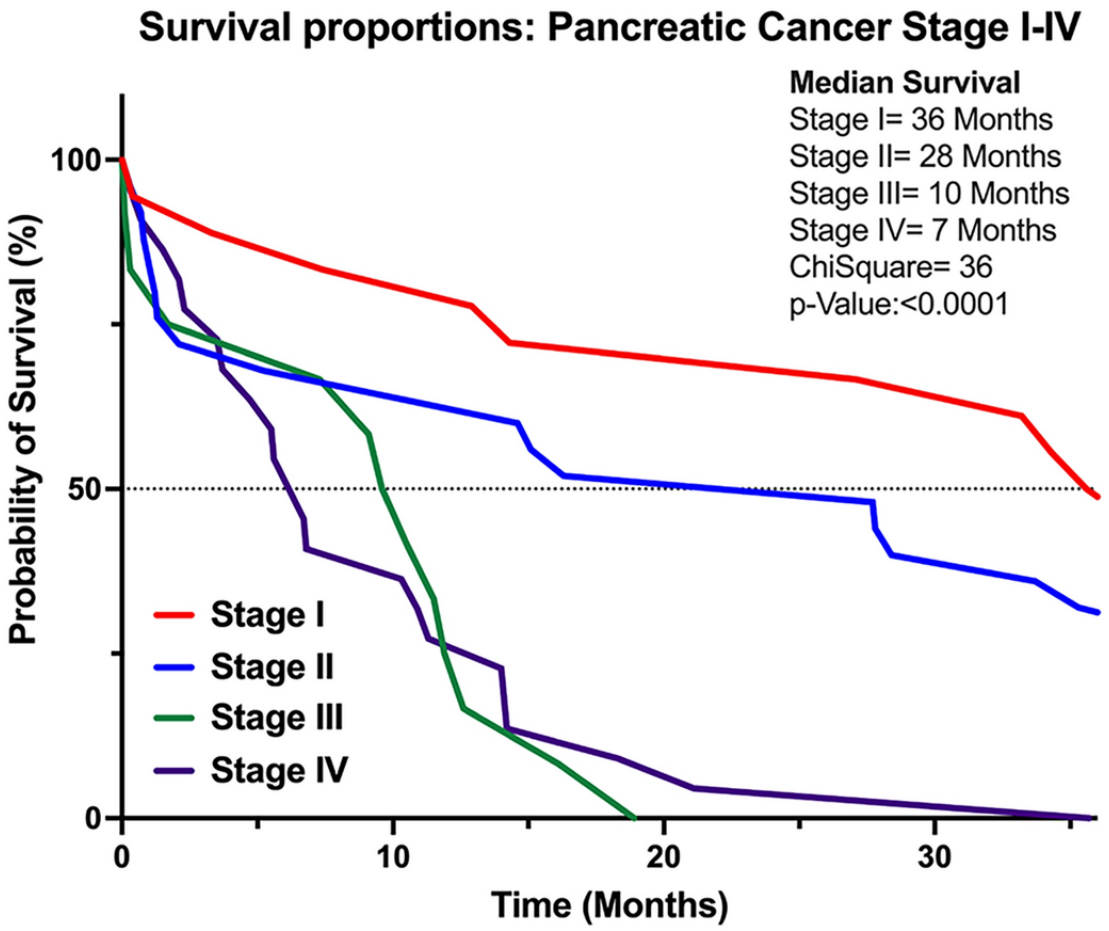
CA Cancer J Clin. 2023 Jan;73(1):17-48.

Max Roser and Hannah Ritchie (2019) - "Cancer". Published online at OurWorldInData.org.

三個導致胰腺癌低存活率的主因



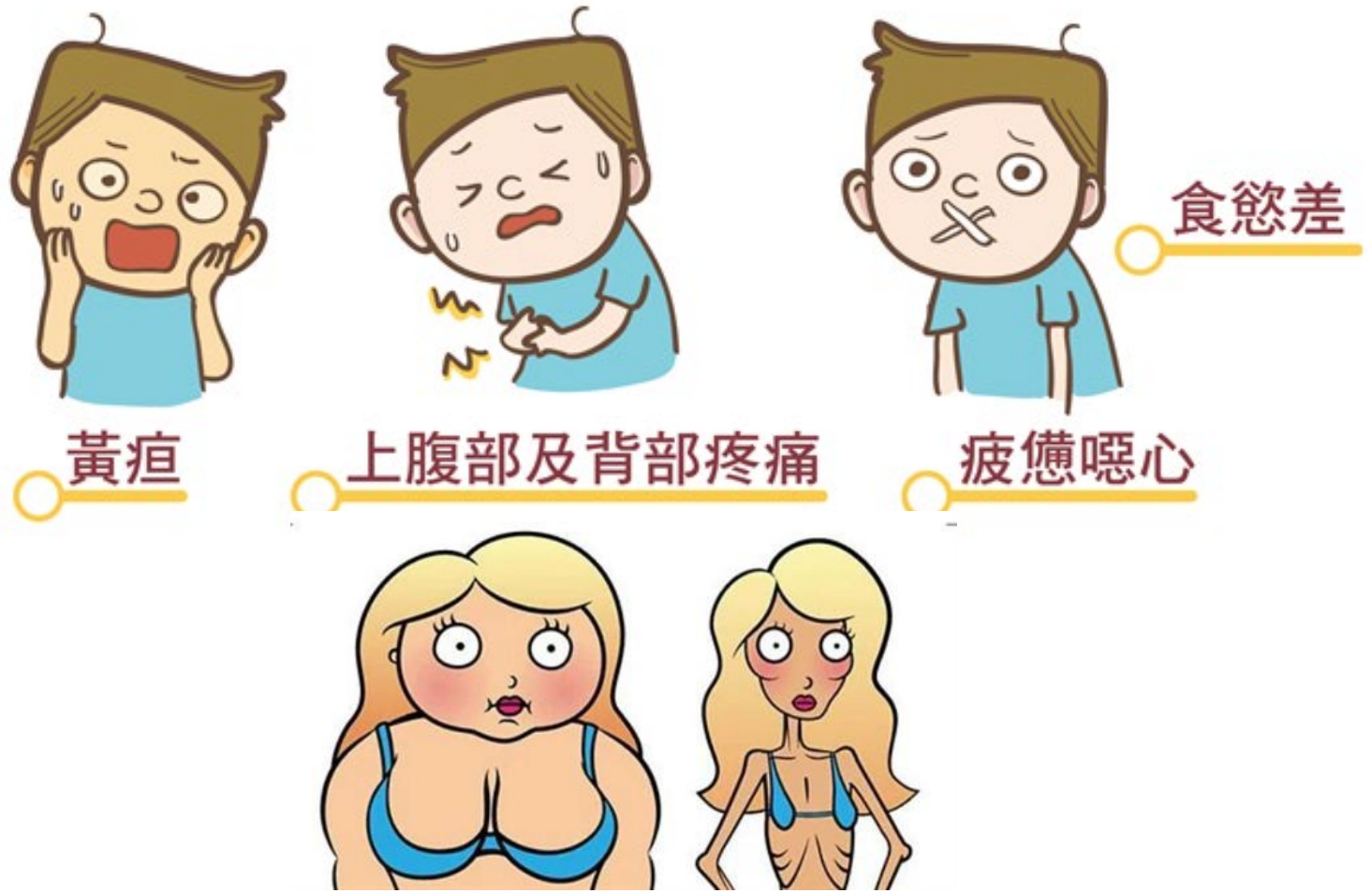
早期發現的胰臟癌存活率較高



Uson Junior, P.L.S., Carvalho, L., Fernandes, M.L.C. *et al.* Neoadjuvant chemotherapy or upfront surgery in localized pancreatic cancer: a contemporary analysis. *Sci Rep* **12**, 13592 (2022). https://doi.org/10.1038/s41598-022-17743-6

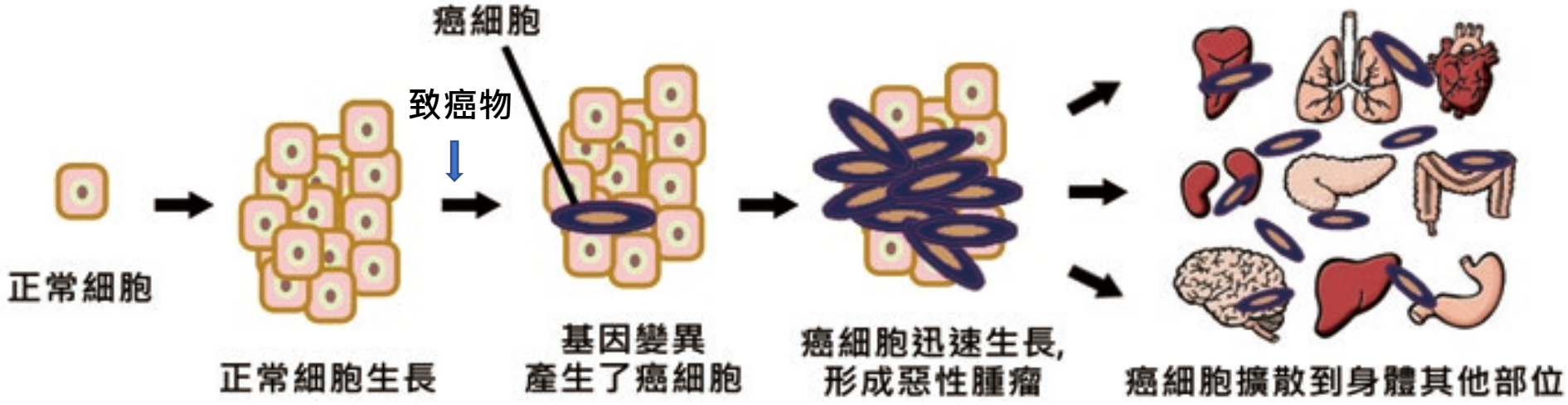
胰腺癌的症狀

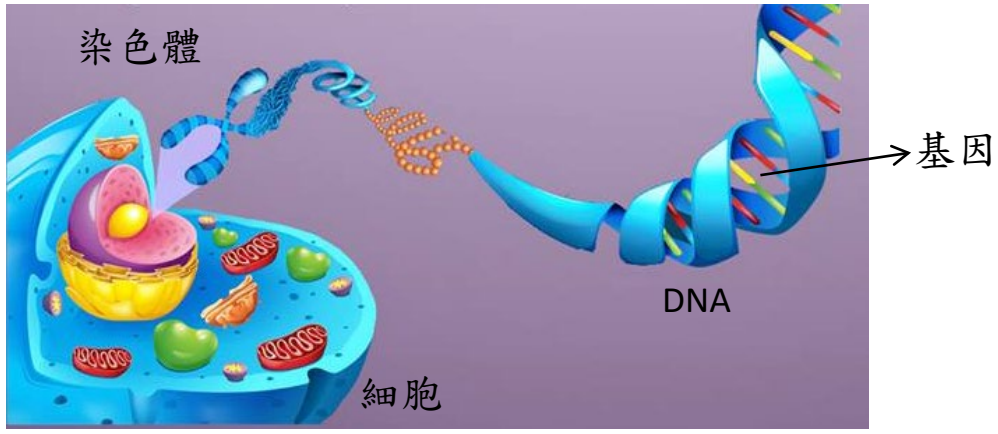
- 早期: 症狀不明顯
- 晚期: 無特異性症狀



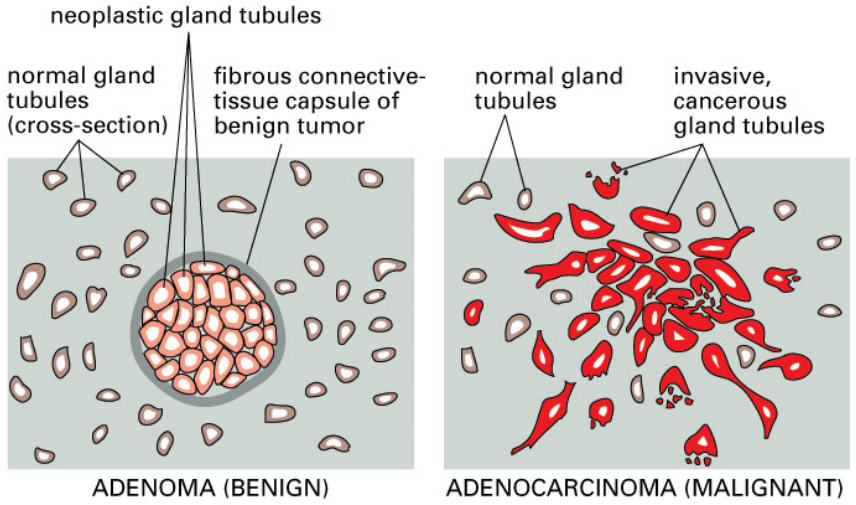
胰腺癌的預防-
了解起源，從飲食調控著手

癌症的起源


癌細胞
致癌物
正常細胞
正常細胞生長
基因變異
產生了癌細胞
癌細胞迅速生長,
形成惡性腫瘤
癌細胞擴散到身體其他部位

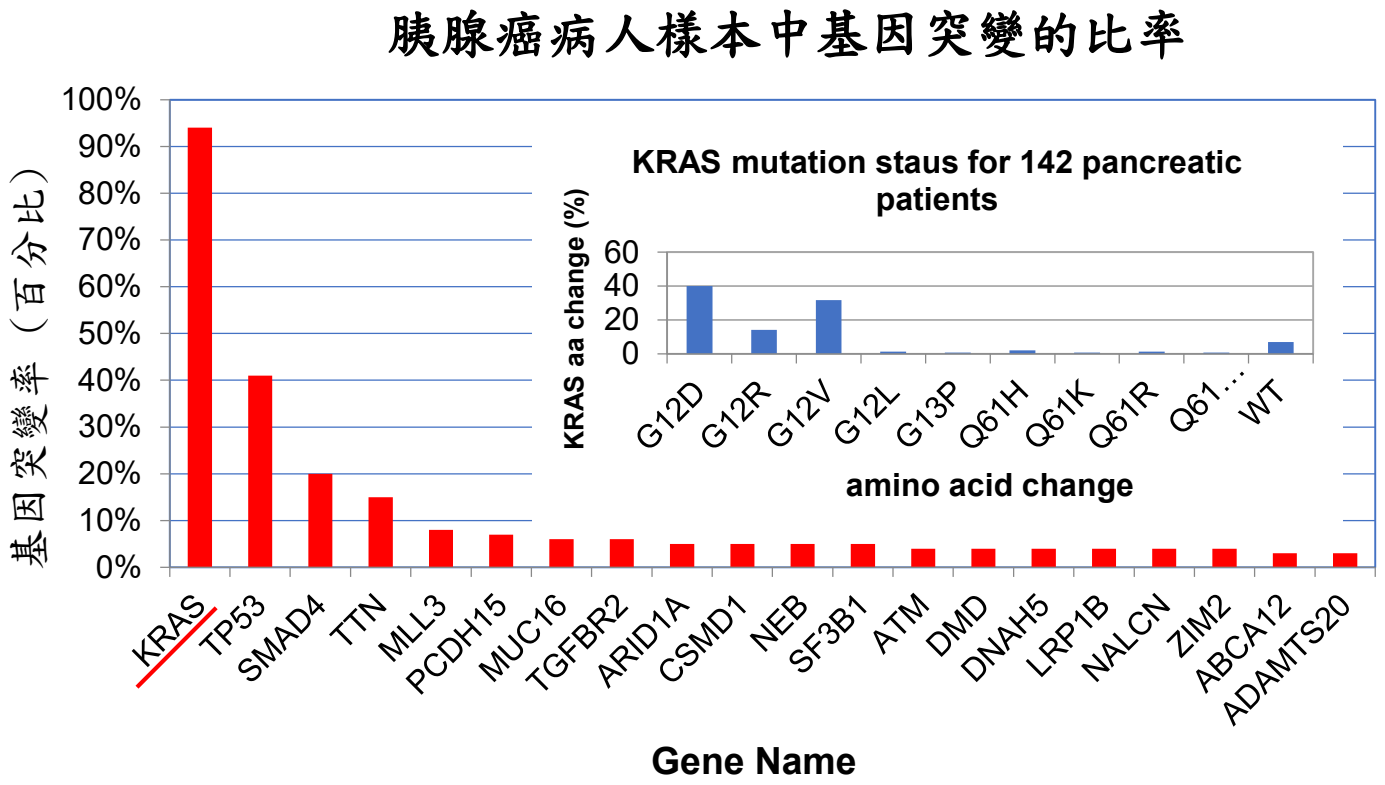

染色體
DNA
細胞

基因


neoplastic gland tubules
normal gland tubules (cross-section)
fibrous connective-tissue capsule of benign tumor
normal gland tubules
invasive, cancerous gland tubules
ADENOMA (BENIGN)
ADENOCARCINOMA (MALIGNANT)

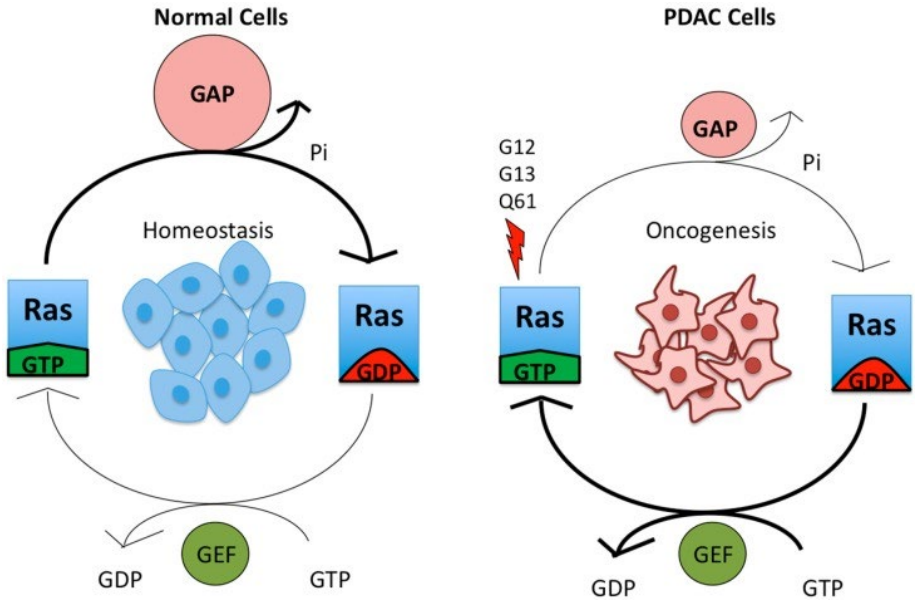
胰腺癌的發展

- 一系列的基因突變
- 超過94%的胰腺癌病人有KRAS基因突變 (使得基因一直活化)
- **KRAS** 基因的活化參與細胞生長與存活

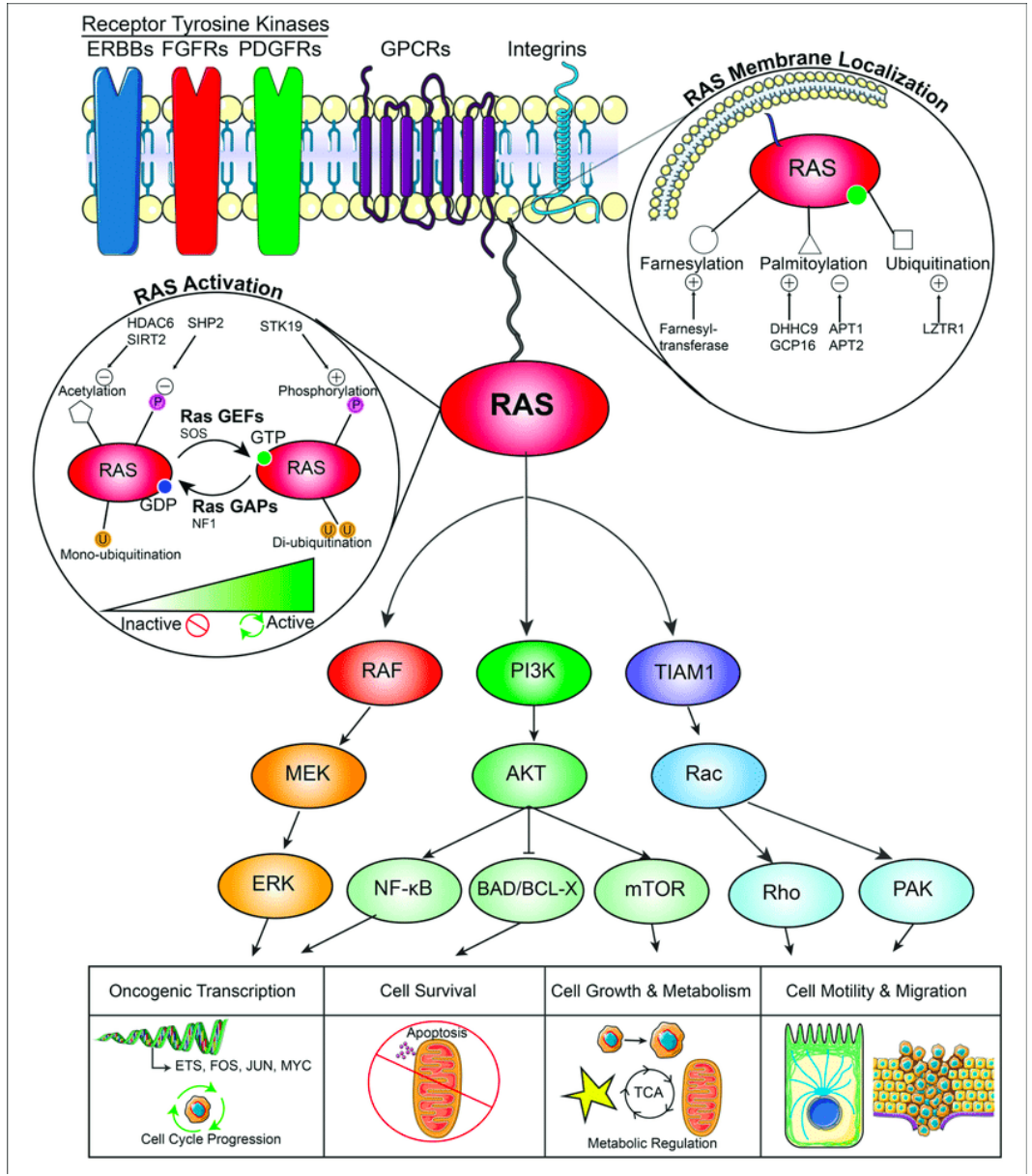


Biankin *et al.*, *Nature*. 2012 Nov 15;491(7424):399-405.

KRAS基因突變是持續與GTP結合的活化狀態

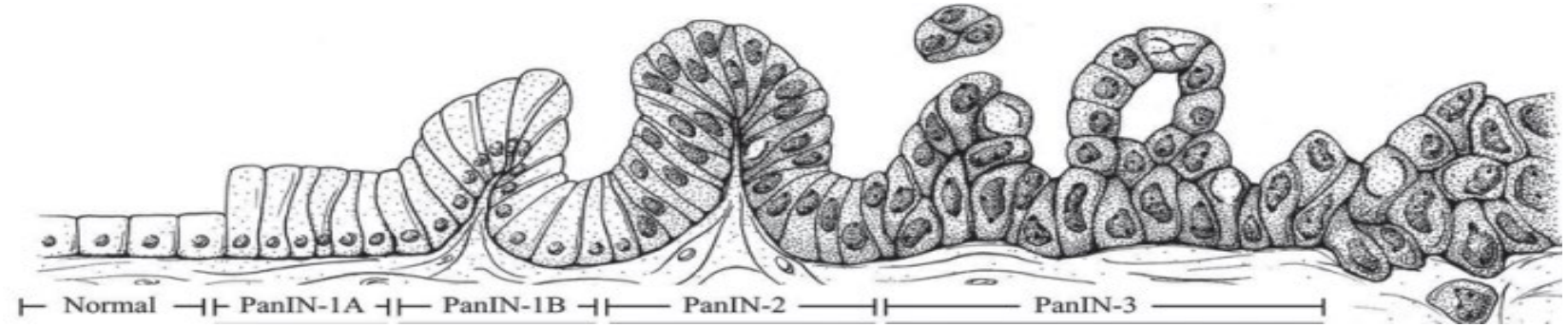


Cancers (Basel). 2016 Apr; 8(4): 45.



Front Oncol. 2019 Sep 24;9:965

KRAS基因突變對於胰腺癌發展是必須的



Normal duct
- Low cuboidal cells
- Single cell layer

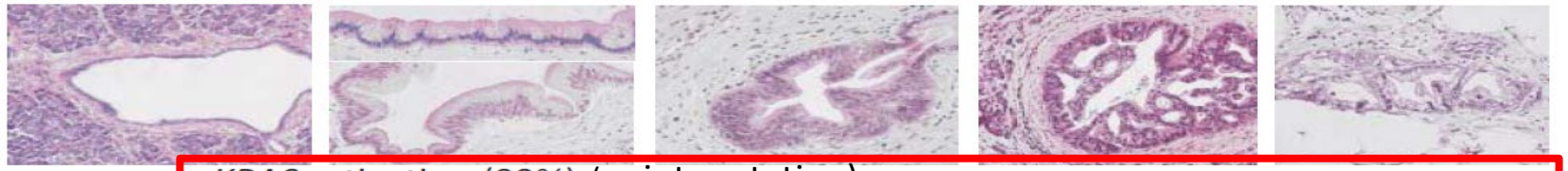
PanIN-1A
- Elongated cells
- Mucin production

PanIN-1B
- Papillary architecture

PanIN-2
- Nuclear abnormalities: e.g. enlargement, some loss of polarity, crowding

PanIN-3
- Budding into lumen
- Severe nuclear atypia
- Mitosis, some abnormal

Adenocarcinoma
- Invasive growth
- Marked stromal reaction (desmoplasia)



KRAS activation (90%) (point mutation)

CDKN2A inactivation (95%) (gene deletion)

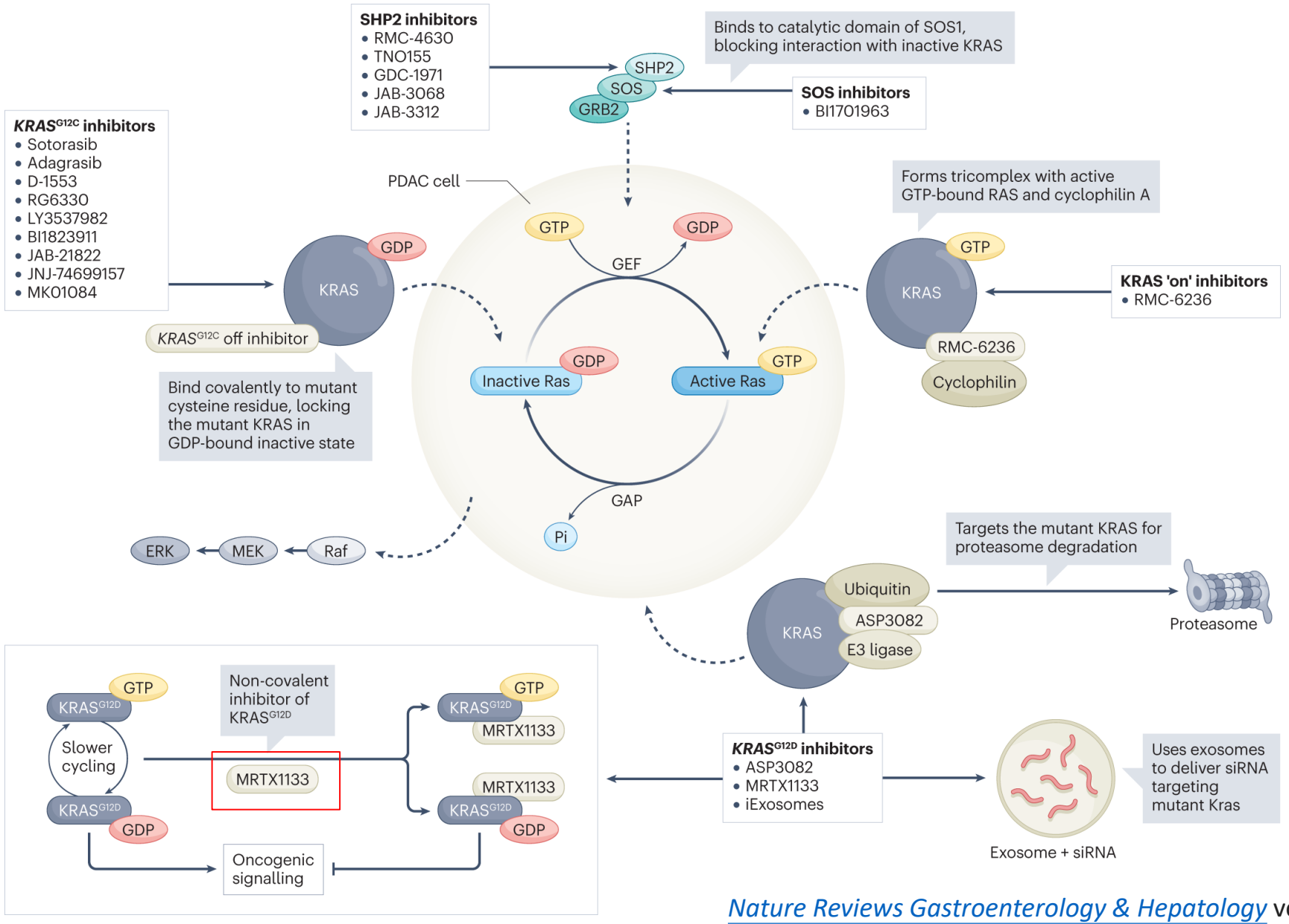
TP53 inactivation (75%) (mutations)
SMAD4 inactivation (55%) (promoter methylation)

Normal epithelium → PanIN-1A → PanIN-1B → PanIN-2 → PanIN-3 → PDAC → Invasion, metastasis

(Koorstra et al., 2008 *Pancreatology*; N Bardeesy , RA DePinho, 2002 *Nat Rev Cancer*), Channing J. Der, 2014 Trends in Biochemical Sciences (review)

胰腺癌中的RAS訊號路徑與治療標靶

KRAS G12D inhibitor
KRAS G12C inhibitor



Nature Reviews Gastroenterology & Hepatology volume 21, pages7–24 (2024)

Q: Why the mutation frequency of KRAS is preferentially high in PDAC?

Two specific aims

- **What kinds of factors are involved in KRAS mutations ?**

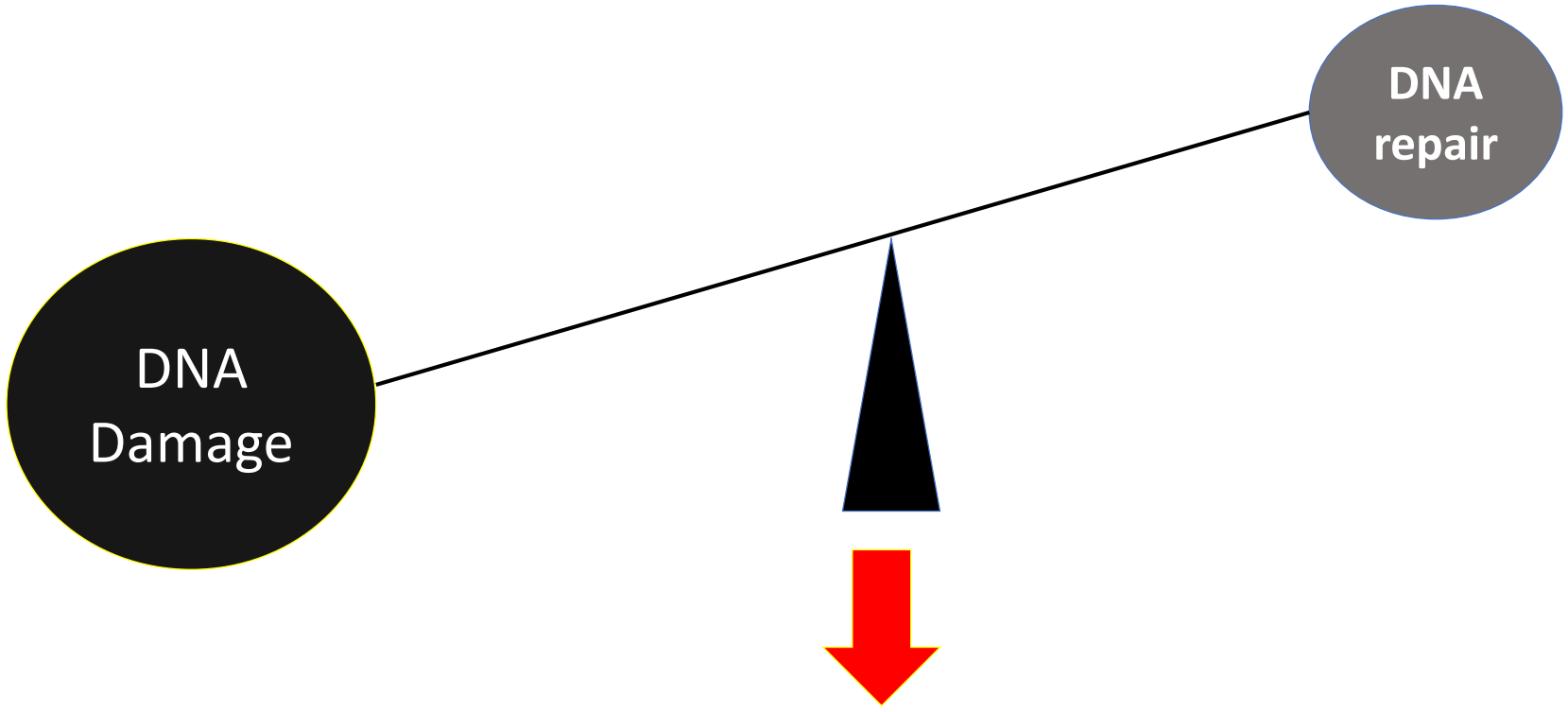
. High Glucose Triggers Nucleotide Imbalance through O-GlcNAcylation of Key Enzymes and Induces KRAS Mutation in Pancreatic Cells.

Hu et al. Cell Metabolism 2019; 29(6):1334-1349. Am J Cancer Res. 2022; 12:1556-1567. Cell Death and Disease. 2022; 13:817 (review).

- **How microenvironment affects/selects the oncogenic mutated KRAS to drive pancreatic intraepithelial neoplasia (PanIN) and PDAC formation ?**
- How microenvironment affects the pancreatic cells with oncogenic KRAS mutation to drive PanIN formation?

Oncogenic KRAS, Mucin 4, and Activin A-mediated Fibroblast Activation Cooperate for PanIN Initiation. Hu et al. Adv. Sci. 2023; 2301240:1-20.

Genes mutations



Mutations arise from unrepaired DNA

在胰臟細胞中，甚麼因子可能造成KRAS基因突變?

胰臟癌危險因子

年齡

Age, over the age of 60

性别

Gender, male

遺傳史

Family history

抽菸

Smoking

糖尿病和高血糖

Type II diabetes and high blood glucose

Overweight and obesity

肥胖過重

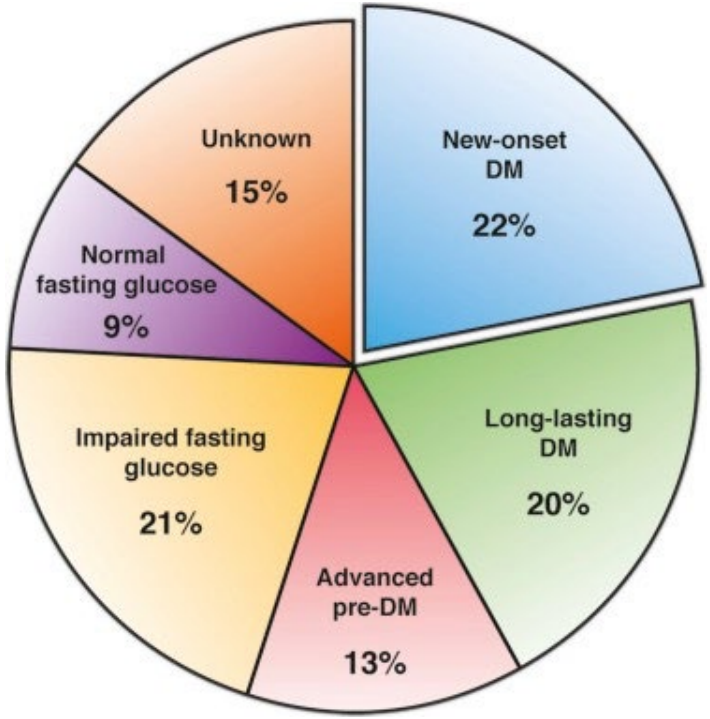
High sugar / high fat diet

高糖高脂的西化飲食

許多臨床上的統計研究顯示**糖代謝的異常**和**胰腺癌**的發生息息相關

Overweight and obesity

High sugar / high fat diet



Gastroenterology Volume 156, Issue 7, May 2019, Pages 2024-2040

Distribution of glycemic status based on fasting blood glucose levels in a population-based PDAC cohort (N = 219).

研究假設

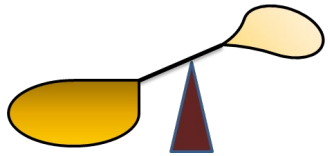
代謝不平衡

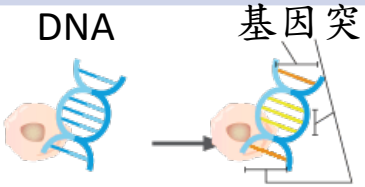
基因組不穩定（DNA 損傷）

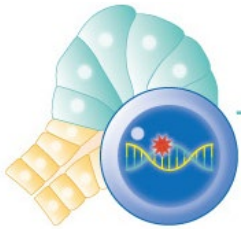
基因突變率 ↑

致癌基因 KRAS突變 ↑

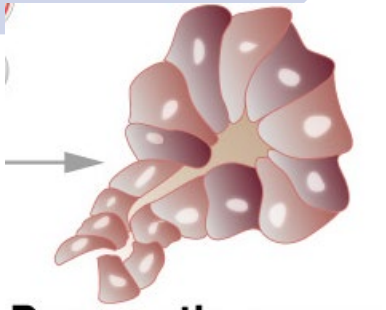
造成胰臟細胞癌化





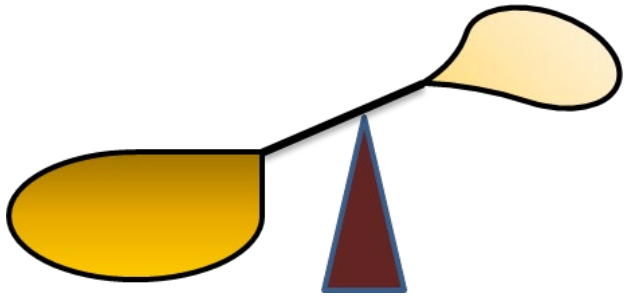


胰臟細胞

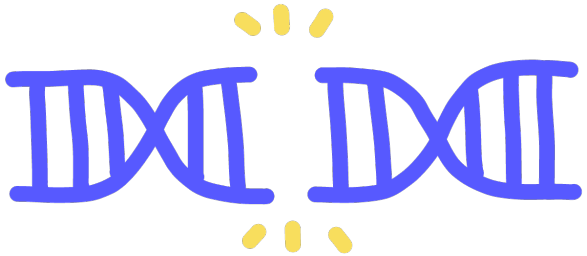


胰臟癌細胞

現象



代謝異常



DNA 損傷

1. 臨床樣本

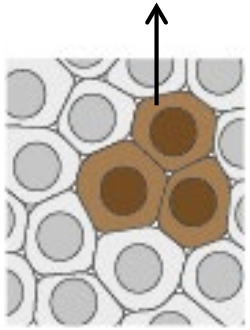
Non-tumor part of pancreatic tissue and intestinal tissues from PDAC patients with/without DM

2. 老鼠樣本

Various tissues from mice fed with chow diet or high sugar/ high fat diet

組織免疫染色

γH2AX: DNA damage and genomic instability marker



組織免疫染色

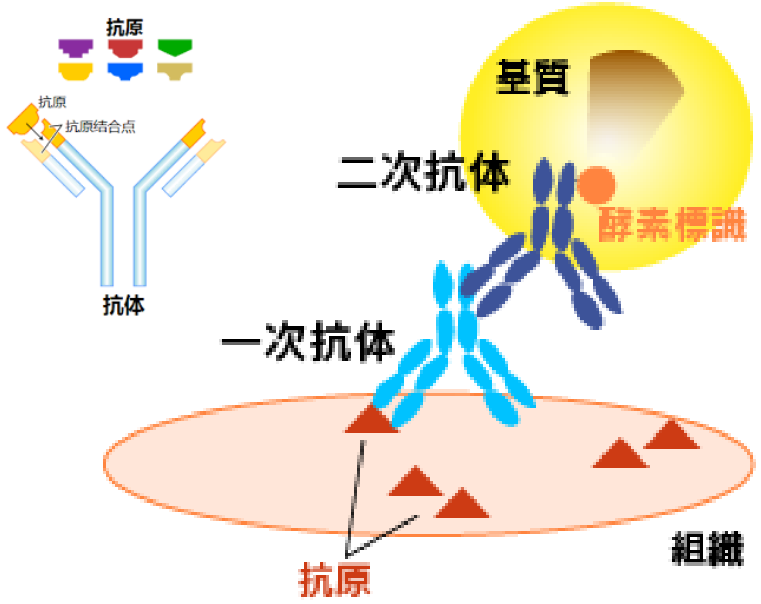
患有糖尿病或沒有糖尿病胰臟癌
病人的非腫瘤組織切片

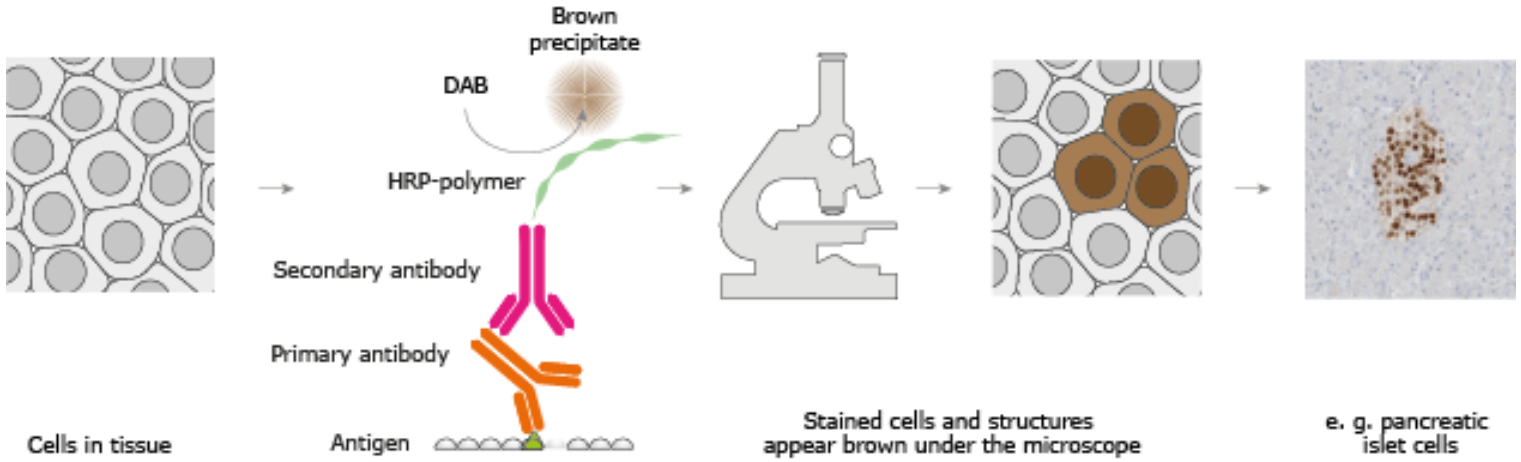
↓

組織免疫染色
anti-γH2AX antibody

↓

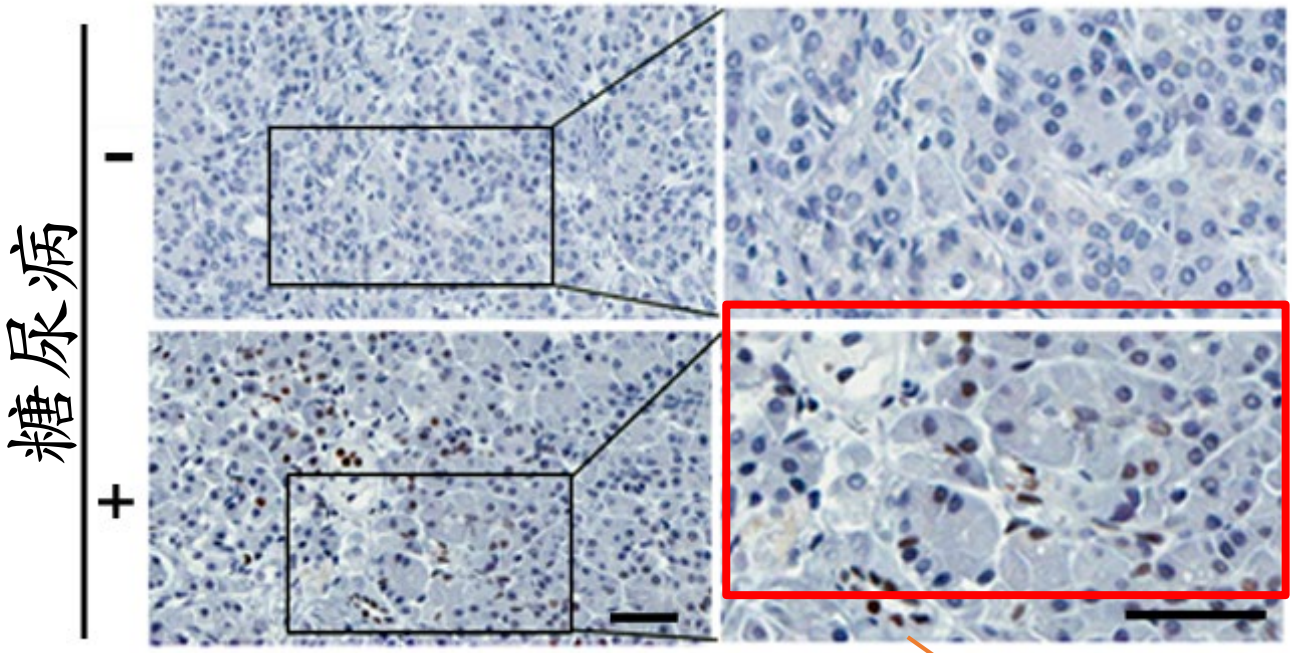
DNA損傷和基因體
不穩定的標記





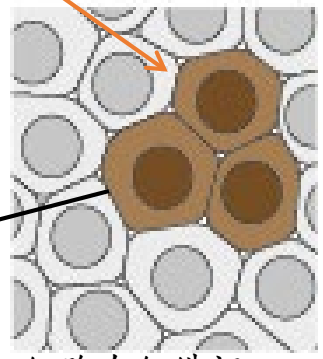
代謝異常容易造成病人胰臟組織的DNA損傷

人類正常的胰臟組織



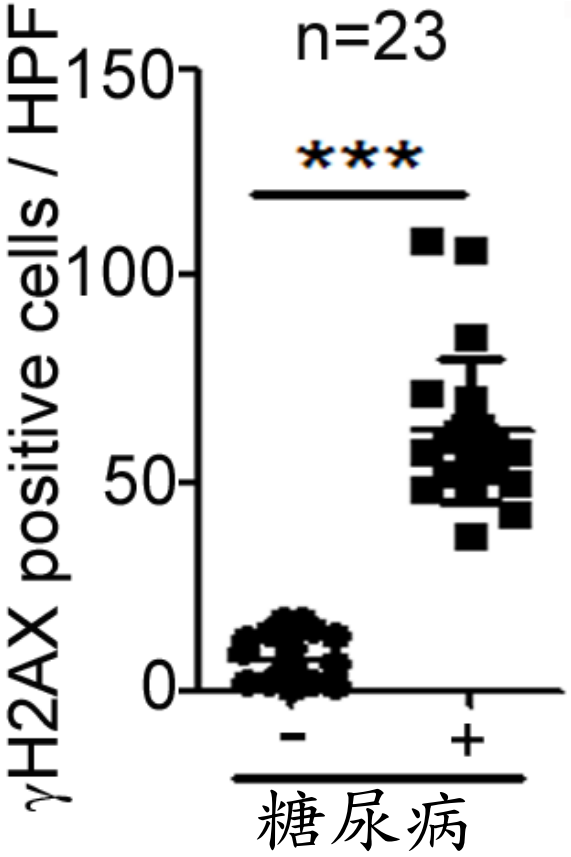
組織免疫染色
anti-γH2AX antibody

DNA損傷和基因體不穩定的標記



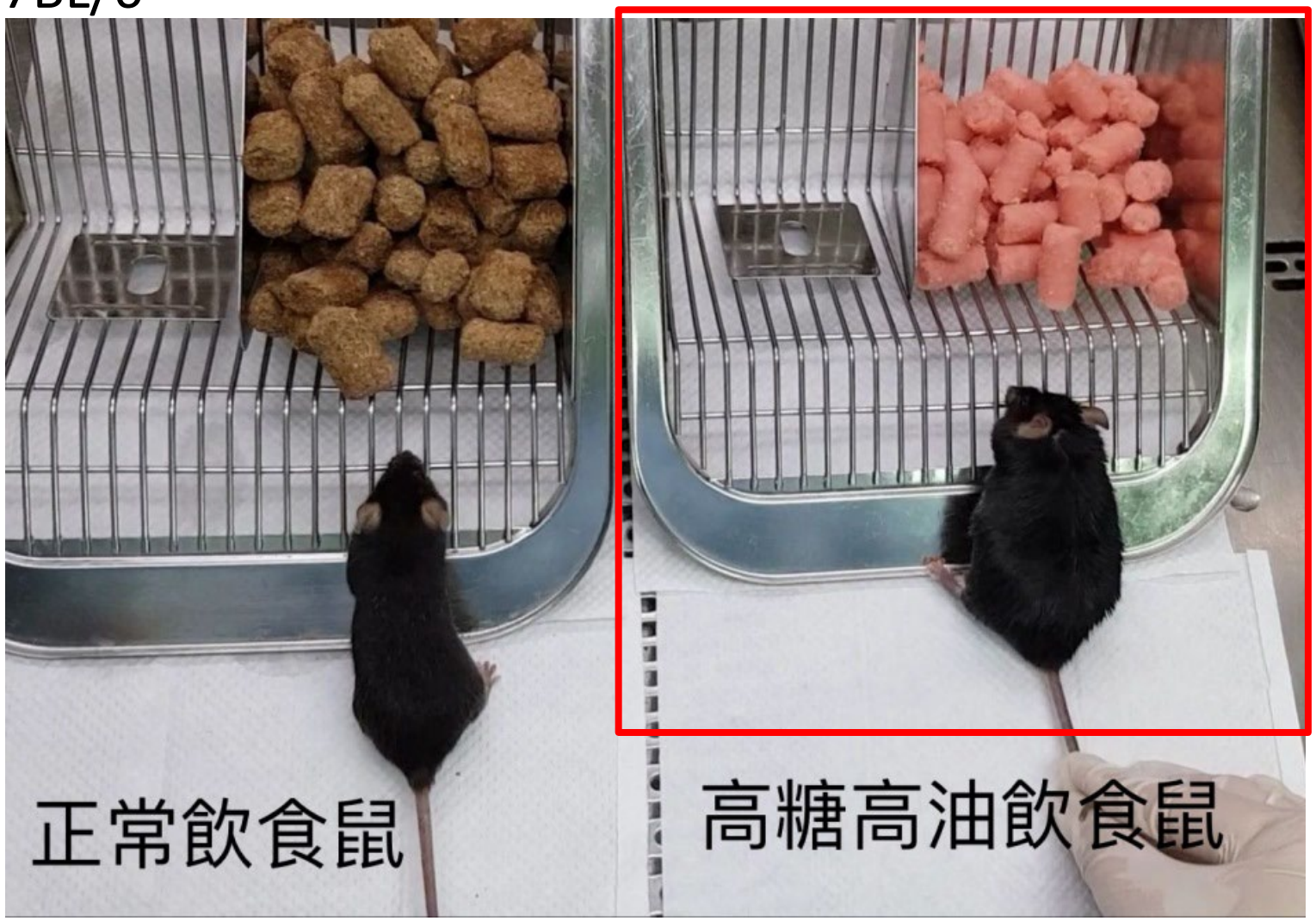
細胞在組織裡

胰臟組織



模擬第二型糖尿病 糖代謝異常的老鼠模式

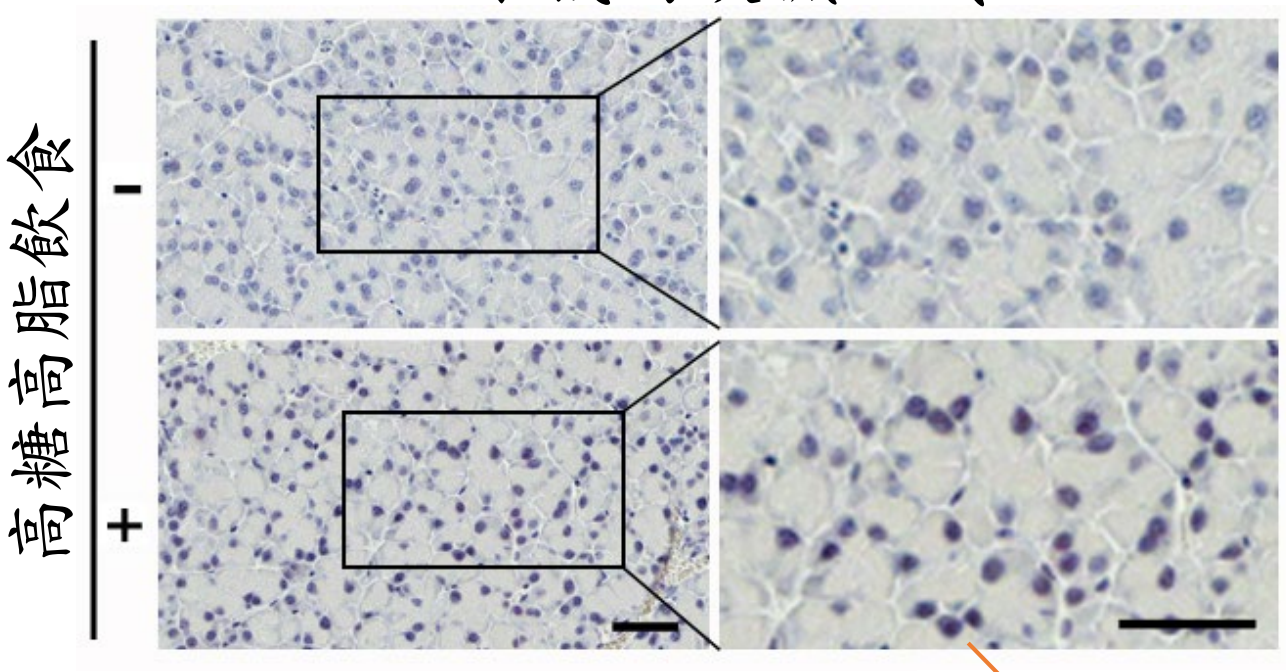
C57BL/6

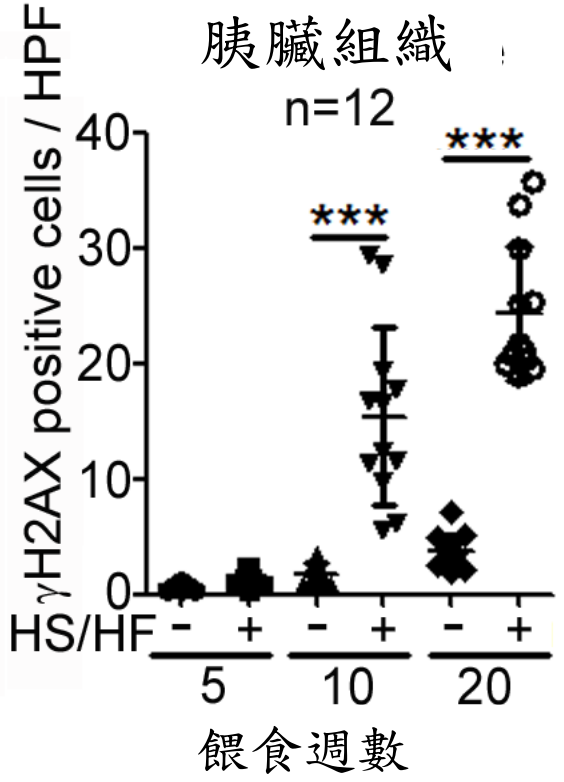


1. 血糖上升2倍
2. 體重增加2-3倍

代謝異常容易造成小鼠胰臟組織DNA損傷

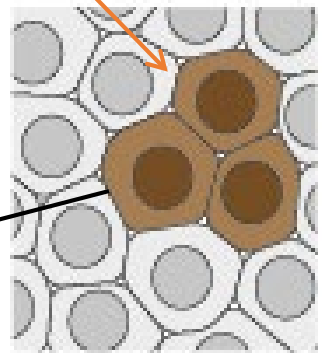
小鼠的胰臟組織





組織免疫染色
anti-γH2AX antibody

DNA損傷和基因體不穩定的標記



其餘小腸、大腸、肝臟、肺臟、腎臟組織DNA損傷皆不顯著

↓

複雜的代謝疾病

↓

在胰臟細胞哪種代謝物異常容易造成DNA損傷?

身體代謝物

碳水化合物 ⟶ 葡萄糖 (glucose)

蛋白質 ⟶ 胺基酸 (amino acid)

麩醯胺酸(glutamine)

是人體中含量最豐富的胺基酸

脂質 ⟶ 脂肪酸 (fatty acid)

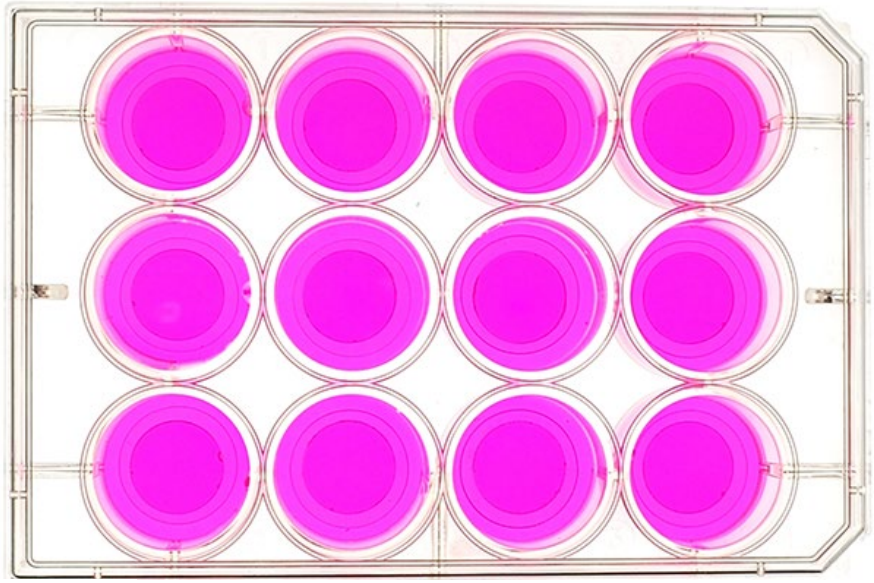
非飽和脂肪酸

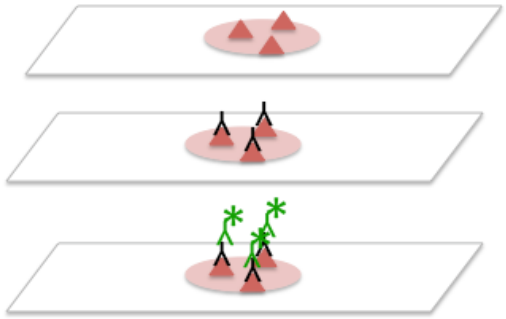
棕櫚酸 (Palmitic acid)

是一種飽和高級脂肪酸，以甘油脂的形式普遍存在於動植物油脂中，在自然界中分布很廣

免疫螢光染色

載玻片

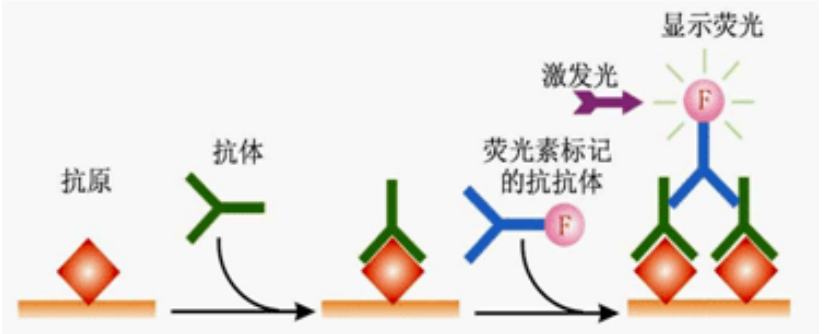




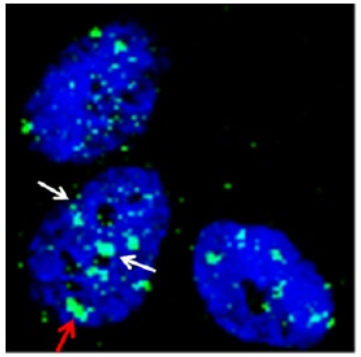
1. Mount sample on slide.

2. Incubate with primary Ab against target antigen.

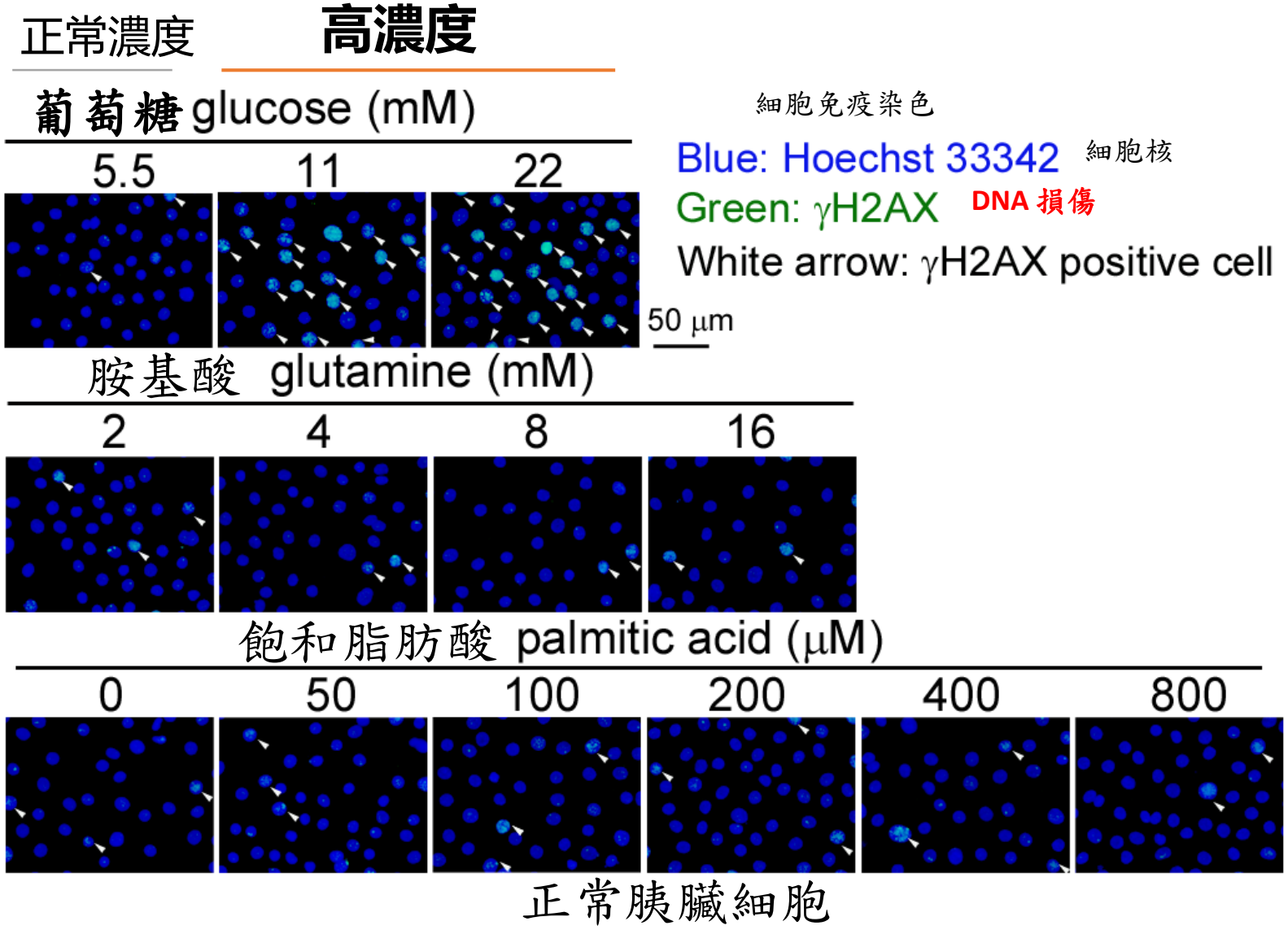
3. Incubate with enzyme, fluorochrome, or gold-linked secondary Ab specific for Fc region of primary antibody.



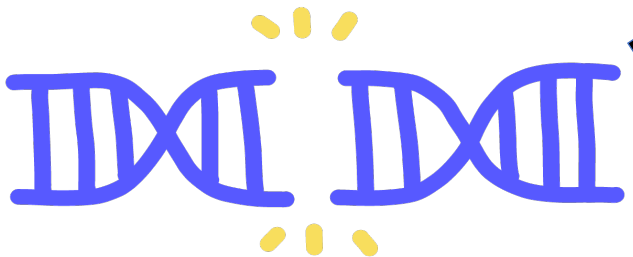
藍色:細胞核
綠色: γH2AX



在胰臟細胞中，葡萄糖容易造成DNA損傷

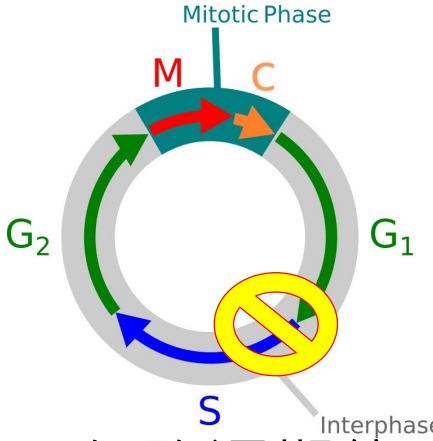


高葡萄糖



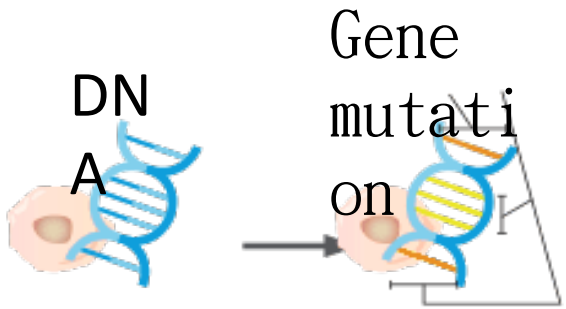
DNA damage

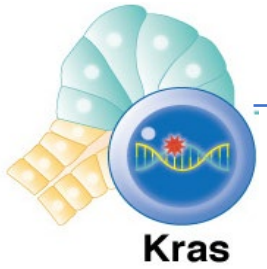
胰臟細胞



細胞週期停止

細胞死亡

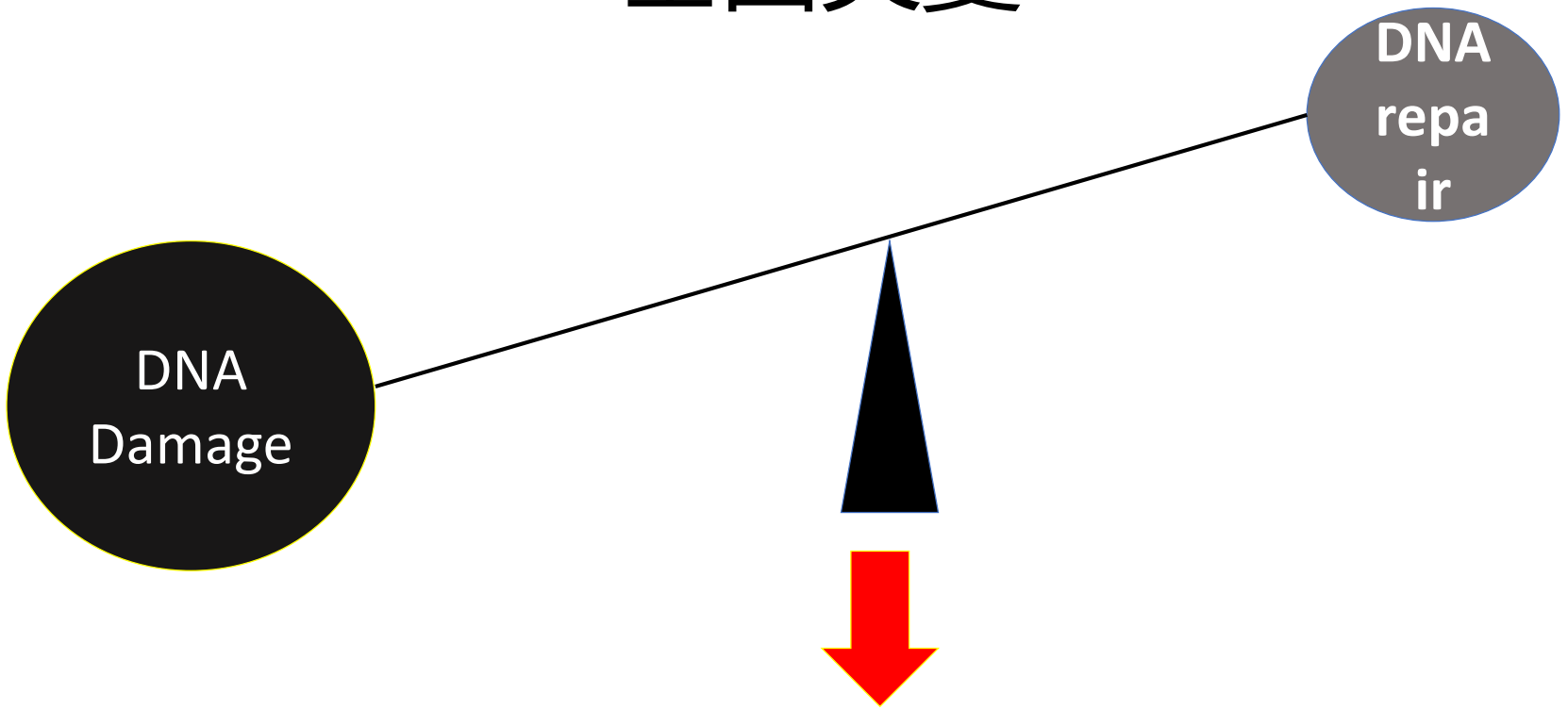




細胞癌化

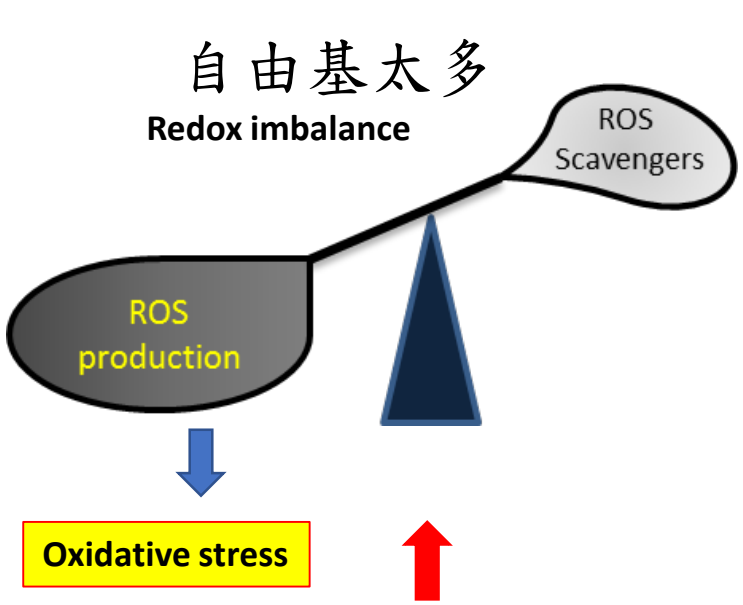
KRAS gene mutations

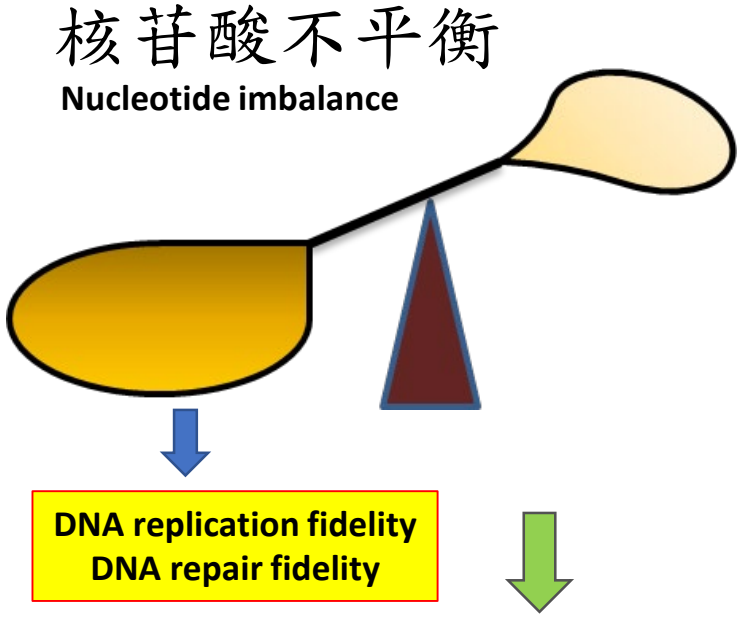
基因突變



Mutations arise from unrepaired DNA

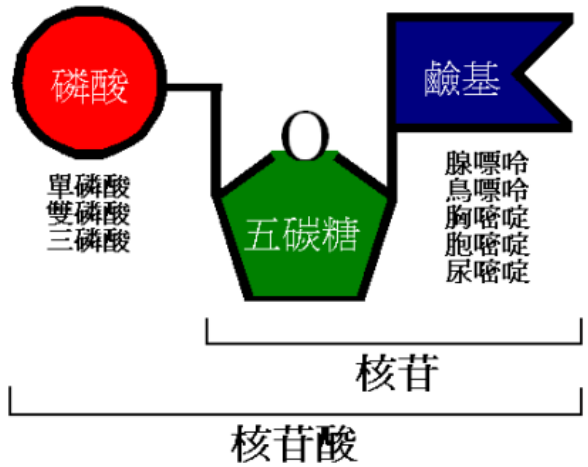
二個主要造成DNA損傷的來源

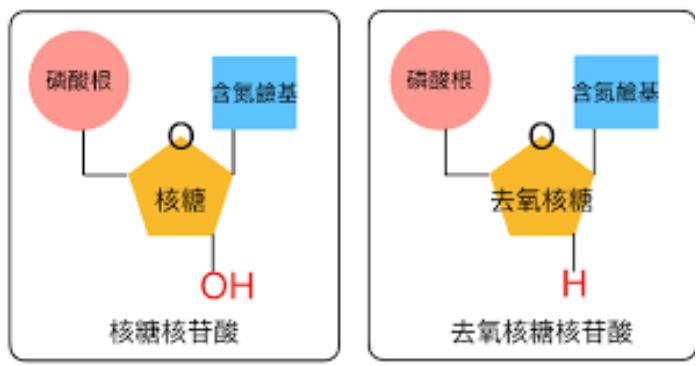




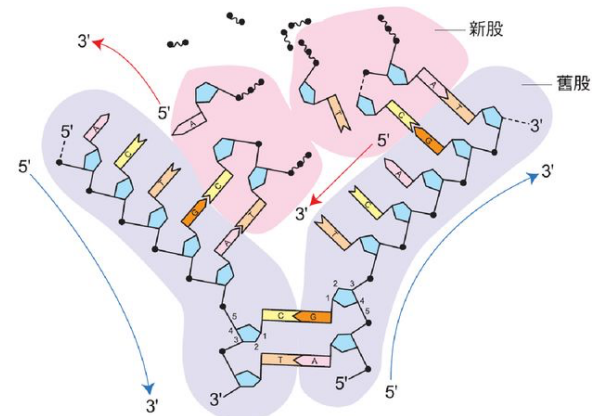
DNA

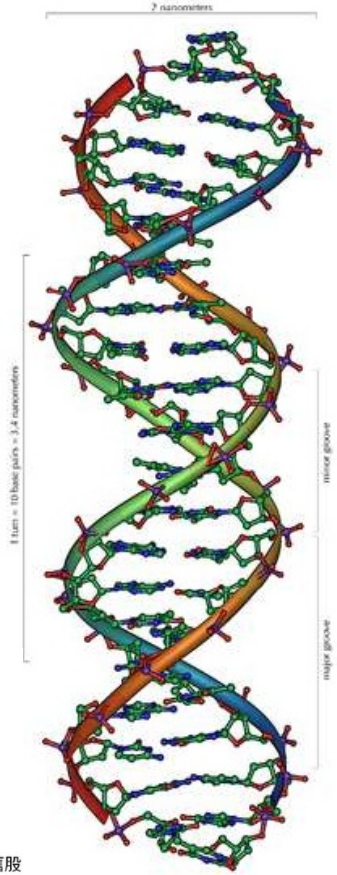
- Deoxyribonucleic acid
- 去氧(脱氧)核糖核酸，是一種長鏈聚合物，藉著四種鹼基組成的遺傳密碼，以引導生物發育與生命機能運作。
- 雙股螺旋(double helix)→





以細胞中的三磷酸去氧核苷酸（dATP、dGTP、dCTP、dTTP）為原料，分別與模板中的含氮鹼基配對（A和T配對，G和C配對）

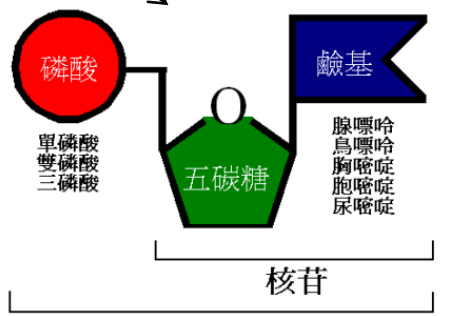


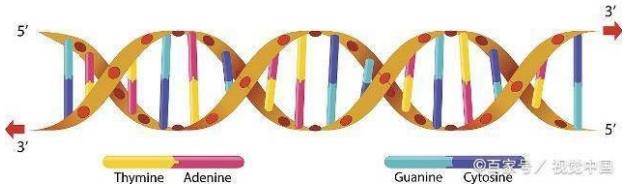


在胰臟細胞，高濃度葡萄糖造成致癌基因KRAS突變的理論

正常葡萄糖

產生能量





胸嘧啶 腺嘌呤 鳥嘌呤 胞嘧啶

DNA複製和修補

正常環境的胰臟細胞

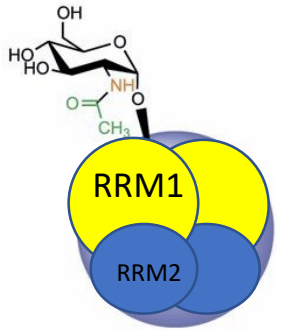
高濃度葡萄糖

PFK活性低

GFAT活性高

產生能量

O-鍵結的蛋白修飾

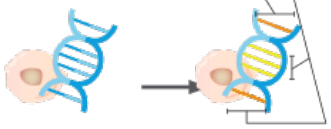


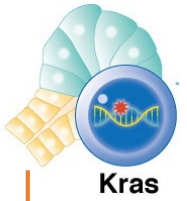
核糖核苷酸還原酶

核苷酸形成減少

DNA複製和修補出問題

DNA 損傷





包含致癌KRAS基因突變

胰臟細胞癌化

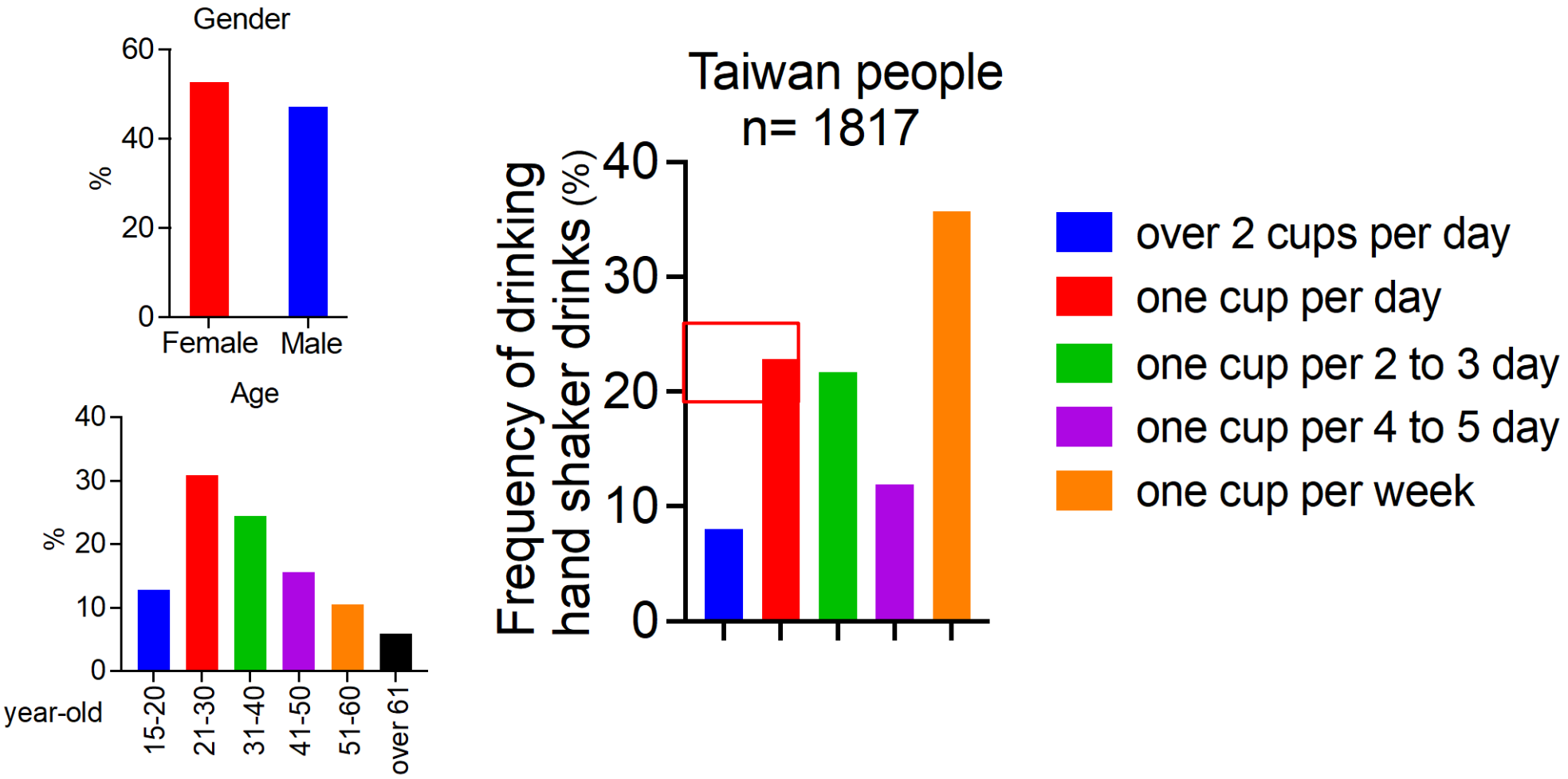
處在高糖環境的胰臟細胞

Hu et al. Cell Metabolism 2019; 29(6):1334-1349.

1. 是否高糖飲料會直接造成胰臟DNA損傷？

2. 是否只要利用乙烯葡萄糖胺普遍增加蛋白質糖化，
就會造成所有器官的DNA都損傷？ 不只是胰臟

超過30%的人每天至少喝一杯手搖飲



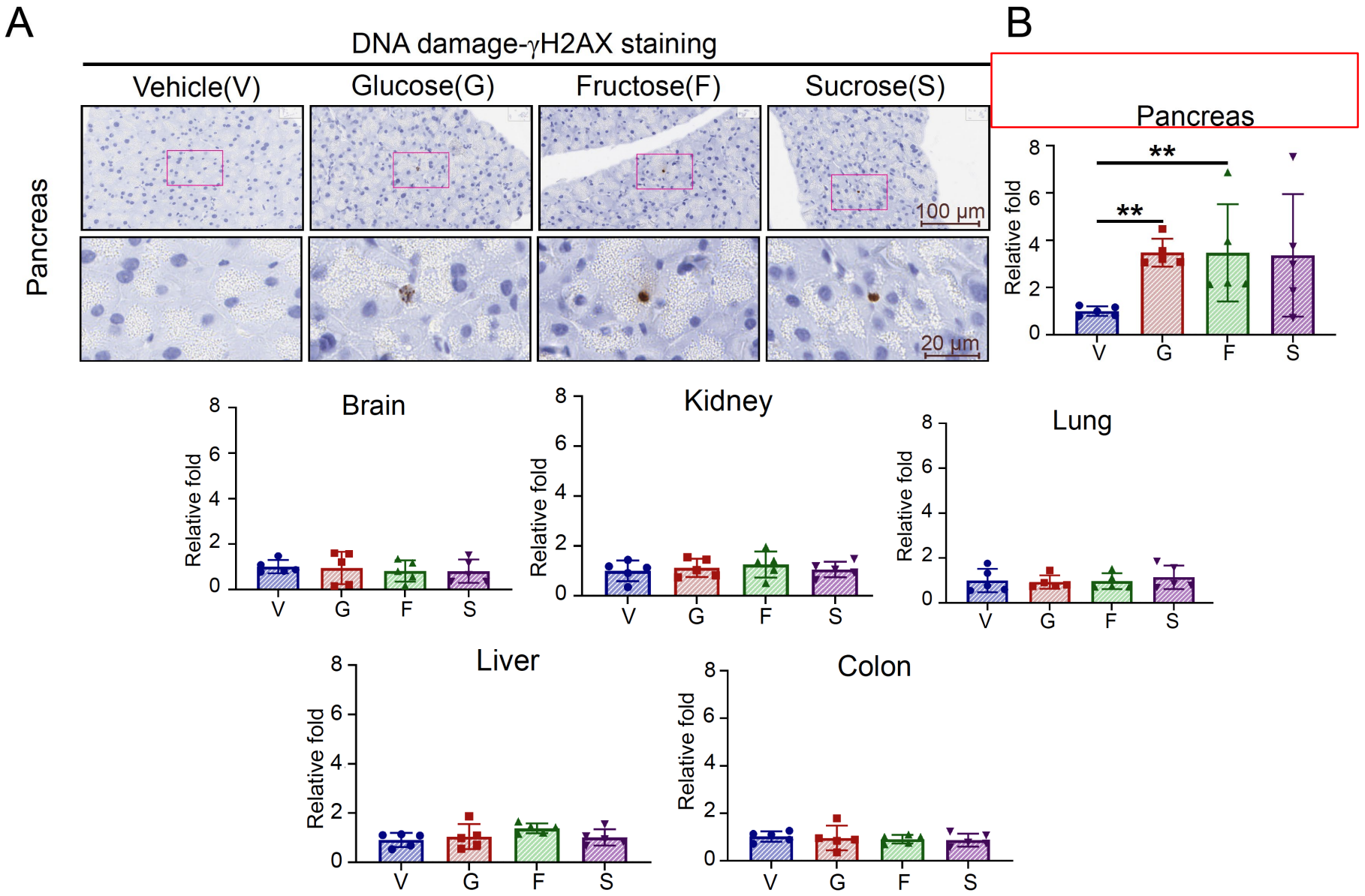
Source: China Marketing Information Service Inc. (CMISI), CMISI 2019 Survey of Brand Preference of Hand Beverage . Frequency of drinking a hand shaker drink in Taiwan. Samples n=1817; survey time: 2019/7/24~2019/8/3

2013~2016衛福部做得到查問卷

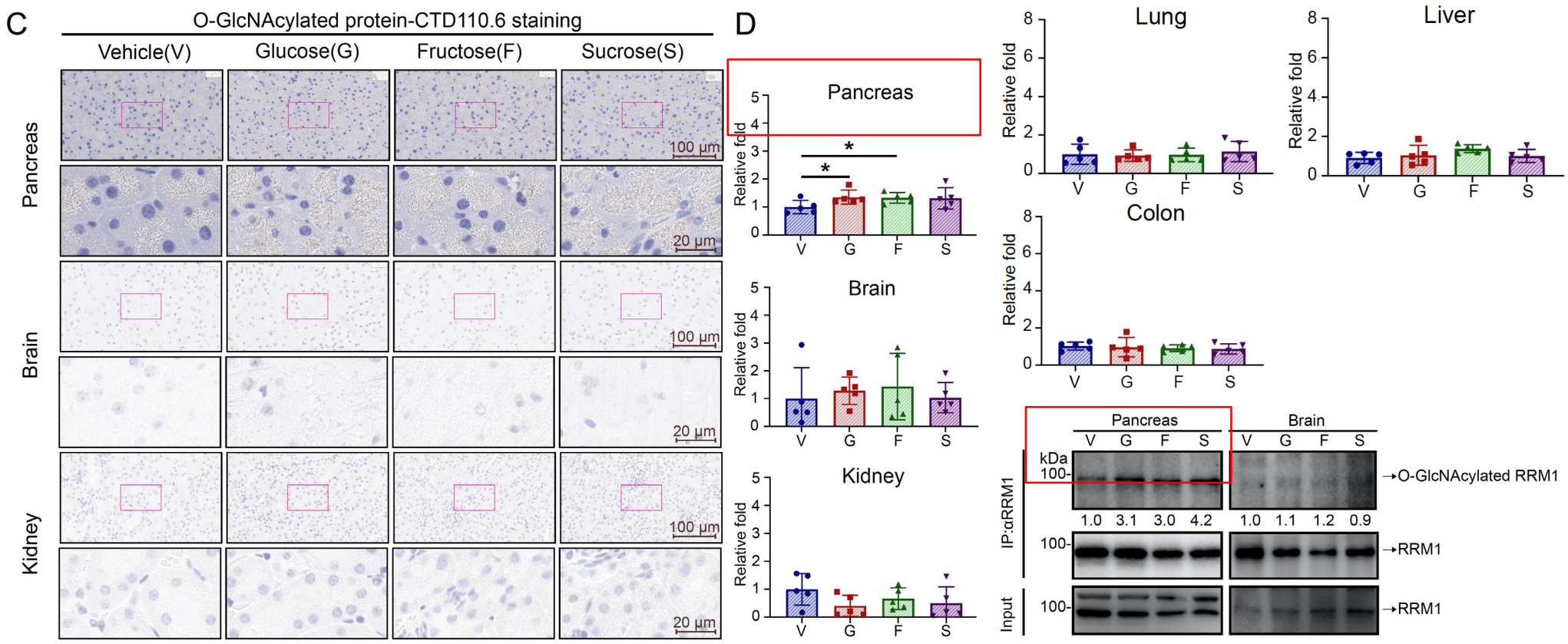
83.6%年齡為19-44歲的台灣成人每天至少飲用一杯含糖飲料，平均一週7.8杯

Health Promotion Administration. Nutrition and Health Survey in Taiwan（衛福部）.
Taipei: NAHSIT (2021). Available at: https://www.hpa.gov.tw/Pages/List.aspx?nodeid=3998

高糖飲料(特別是葡萄糖和果糖)只在胰臟造成較高的DNA損傷



高糖飲料在胰臟組織造成蛋白質糖化和RRM1糖化修飾和在胰臟細胞中一樣

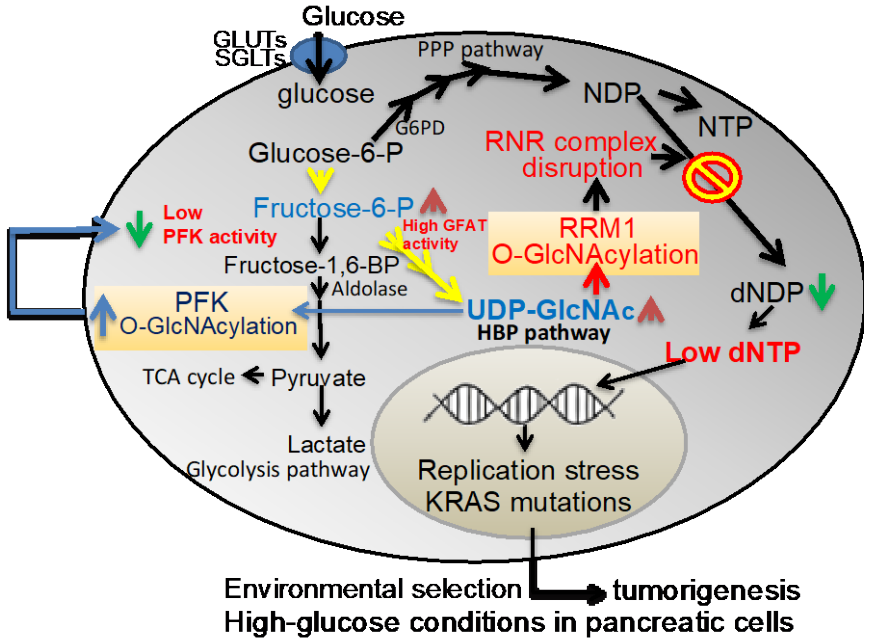


The mechanism of high sugar on inducing DNA damage in the pancreatic tissue is similar to the high glucose effect in

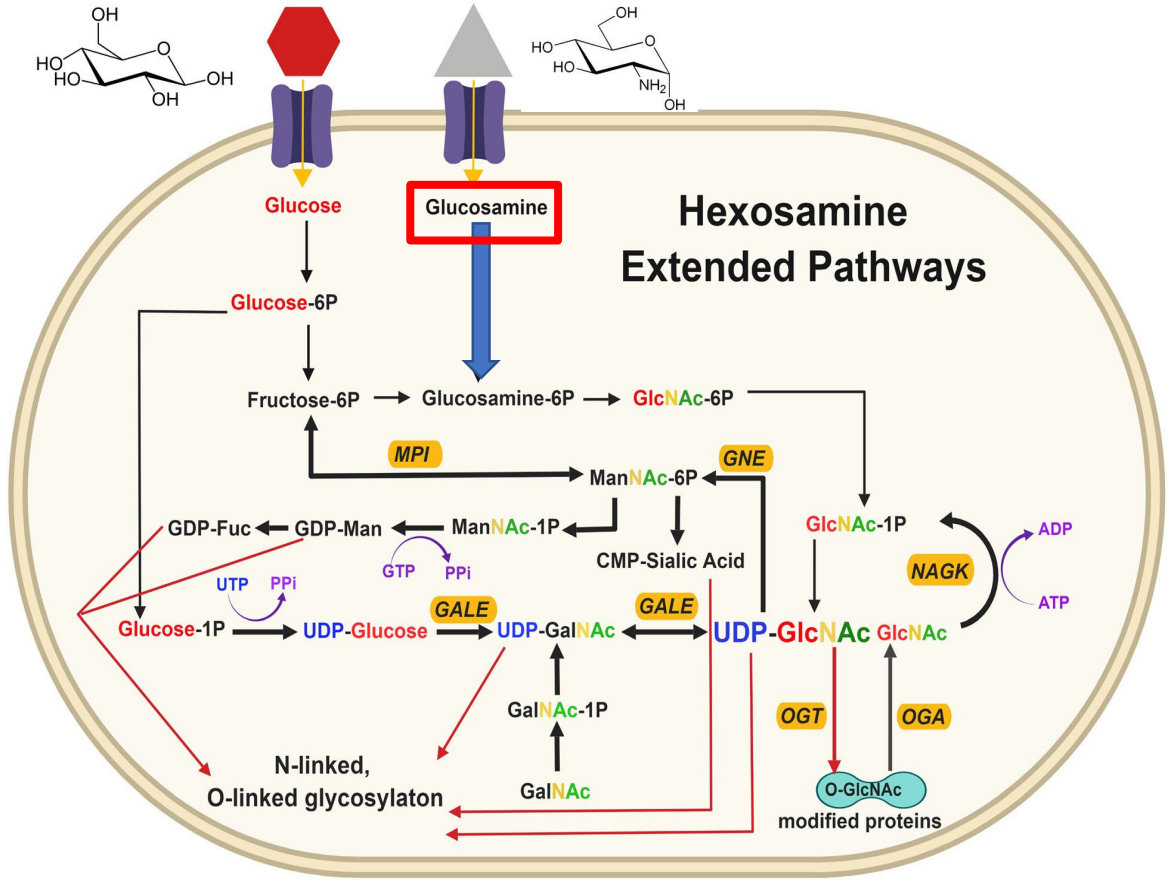
是否只要利用乙烯葡萄糖胺普遍增加蛋白質糖化，就會造成所有器官的DNA都損傷？ 不只是胰臟

N-acetyl Glucosamine (維骨力的一種）, which is sold as a healthy food and beverage additive

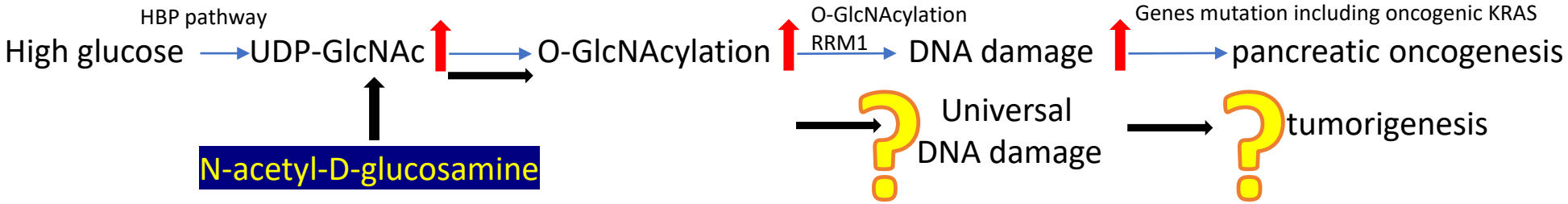
乙烯葡萄糖胺(N-acetyl-D-glucosamine)對於誘發基因組不穩定的可能影響



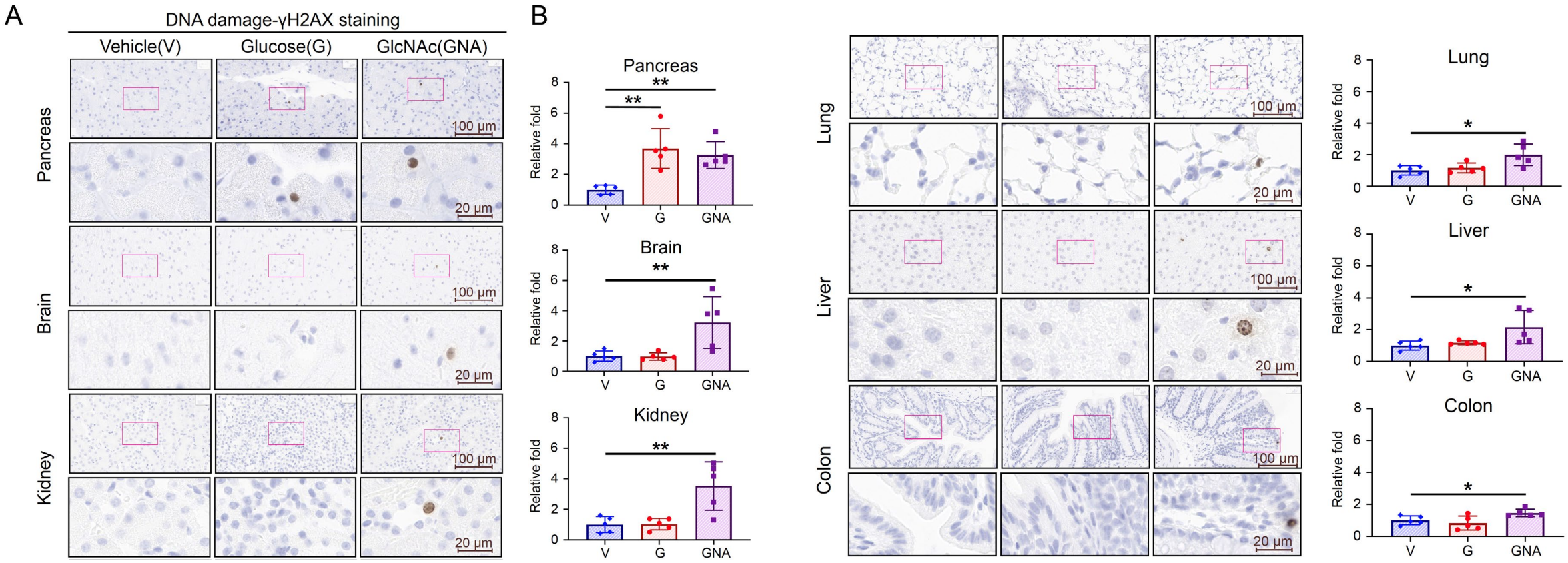
Hu et al. Cell Metab. 2019 Jun 4;29(6):1334-1349.



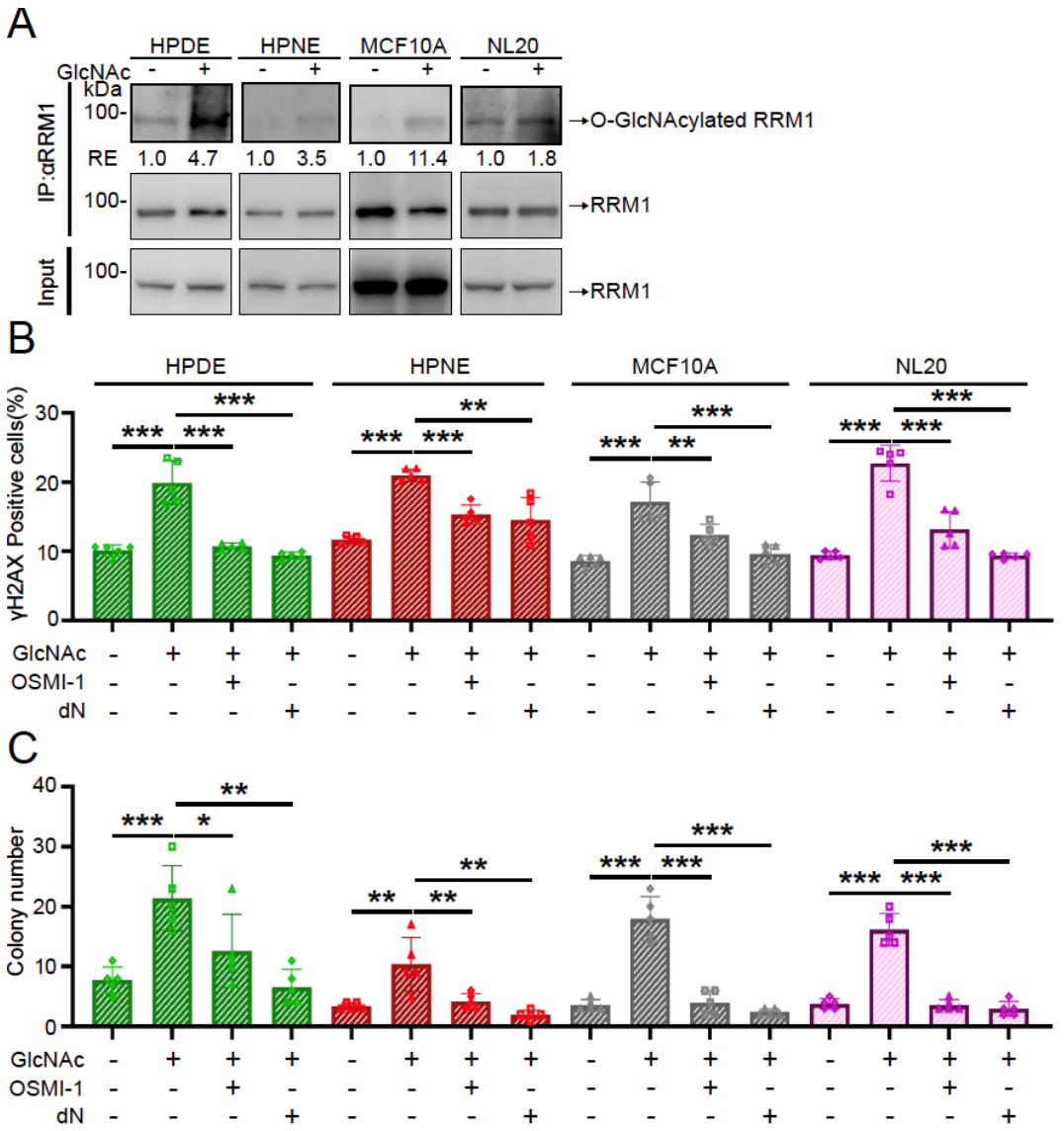
Akella, N. M., (2019). BMC biology, 17(1), 52.



高糖飲料和高乙烯葡萄糖胺對於造成器官中的DNA損傷有差異影響



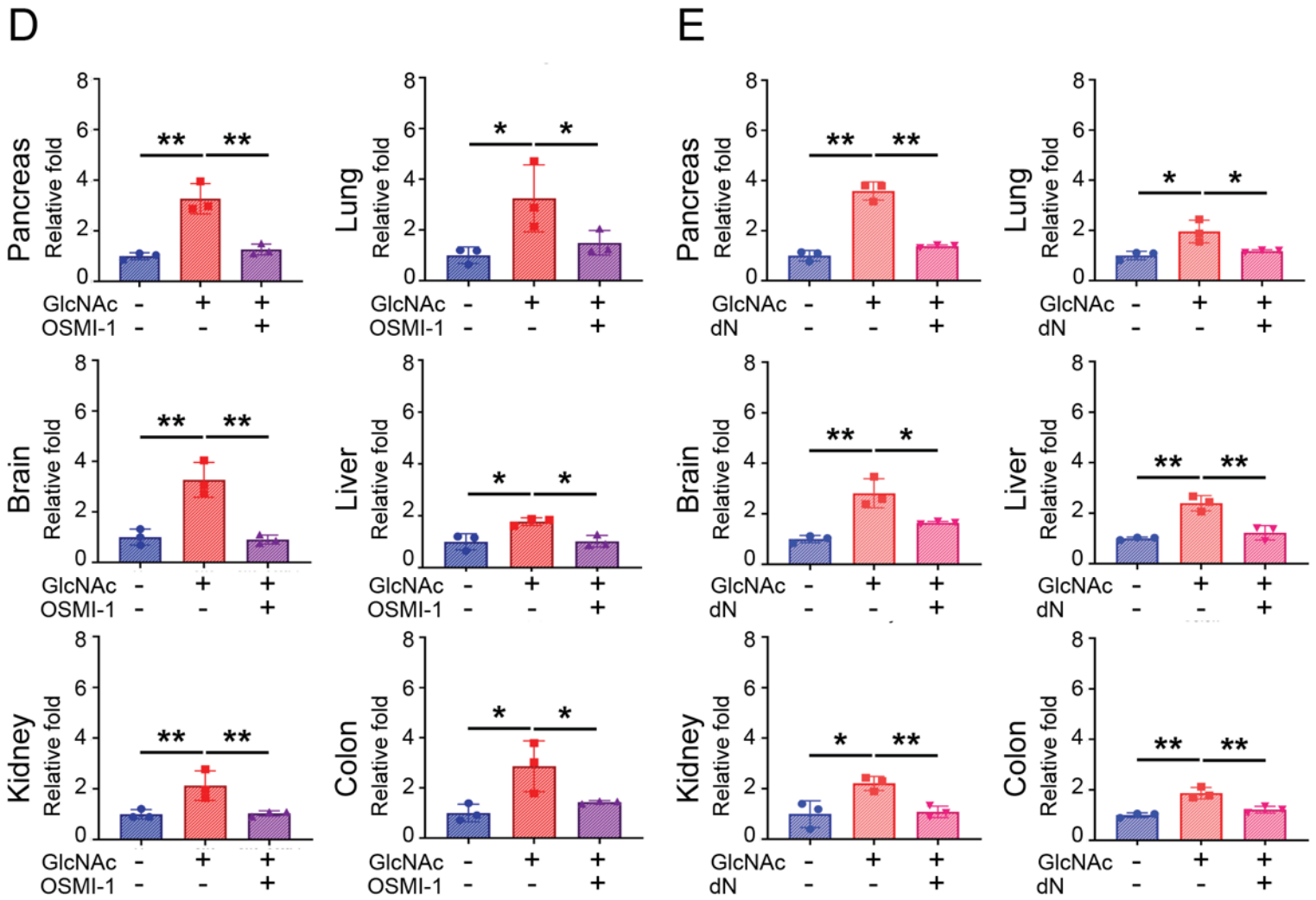
葡萄糖胺在各細胞引發的作用和高葡萄糖在胰臟細胞的作用相似



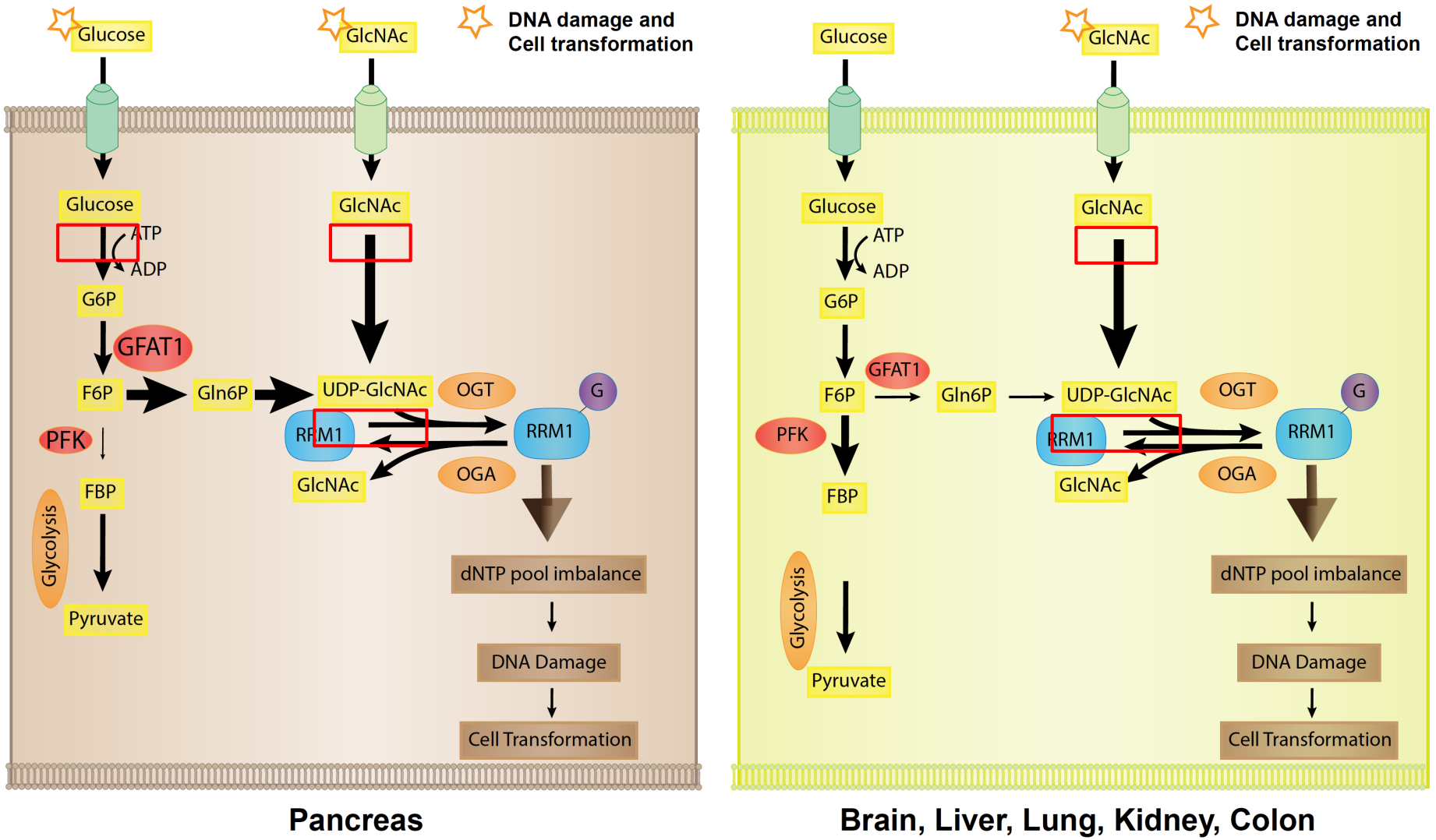
dN:
increase cellular dNTP

OSMI-1: a potent OGT Inhibitor, reduce O-GlcNAcylation

抑制蛋白質O-連結糖化和補充4種核苷可以抑制葡萄糖胺在六種器官中造成的DNA損傷

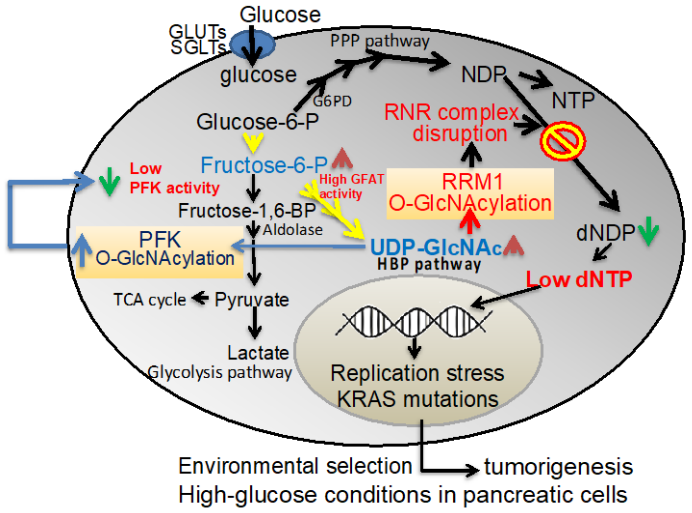


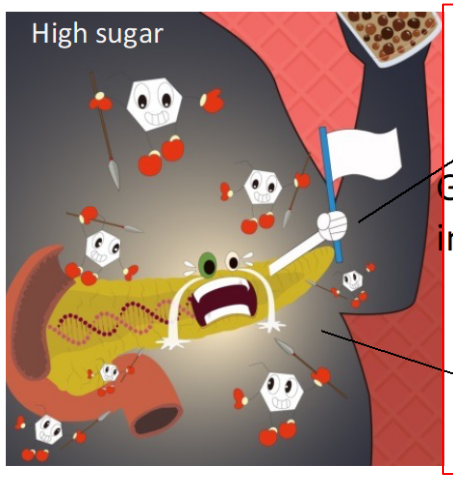
高葡萄糖和高葡萄糖對於各種組織DNA損傷和癌化的差異性作用

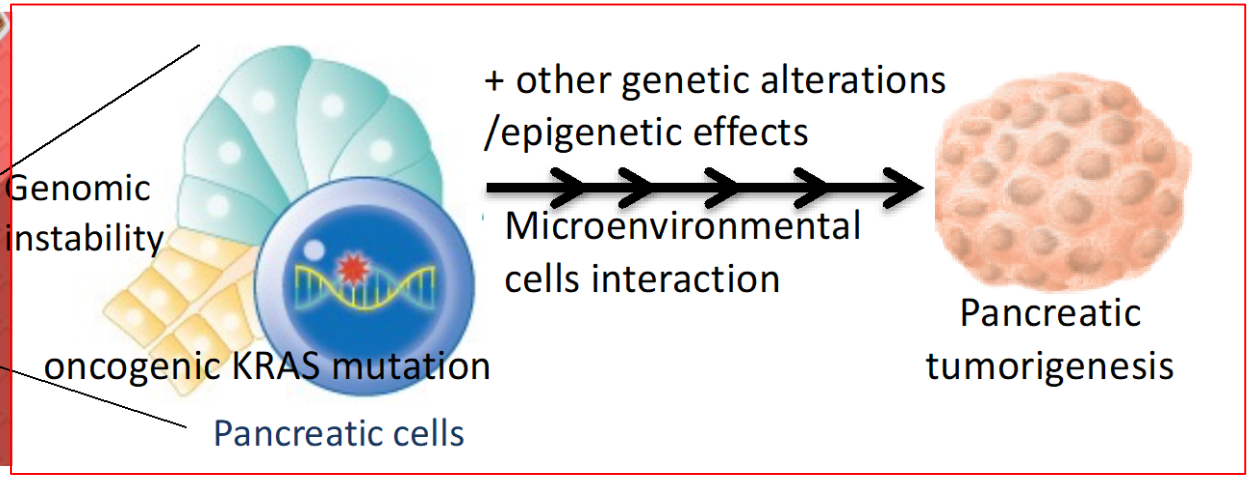


Hsu et al. Am J Cancer Res. 2022; 12:1556-1567

糖代謝和微環境相互作用促進胰腺癌發展







Prevention Cancer initiation → **Why KRAS mutation is preferentially high in PDAC ?**

↓ Selection

How does gene-microenvironment interaction affect/select the mutated KRAS to drive PDAC formation?

胰臟癌前病變組織,胰臟上皮內瘤(PanIN)

KRAS activation (90%) (point mutation)

CDKN2A inactivation (95%) (gene deletion)

(Koorstra et al., 2008 *Pancreatology*; N Bardeesy , RA DePinho, 2002 *Nat Rev Cancer*), Channing J. Der, 2014 Trends in Biochemical Sciences (review)

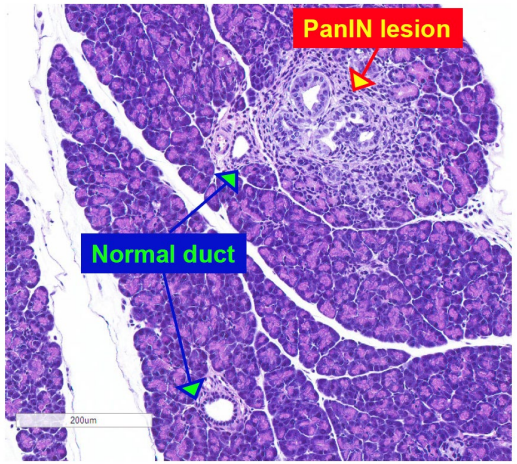
Mutant KRAS-mediated PanIN initiation

TP53 inactivation (75%) (promoter methylation)

SMAD4 inactivation (55%)

Normal epithelium → PanIN-1A → PanIN-1B → PanIN-2 → PanIN-3 → PDAC → Invasion, metastasis

4-week-old *Pdx1-Cre; Kras*$^{G12D/+}$ (PK) mice (M784)



Pancreatic cells with oncogenic KRAS mutation

Intrinsic effect

External effect

Metabolite reprogramming

Genomic instability
Epigenetic regulation

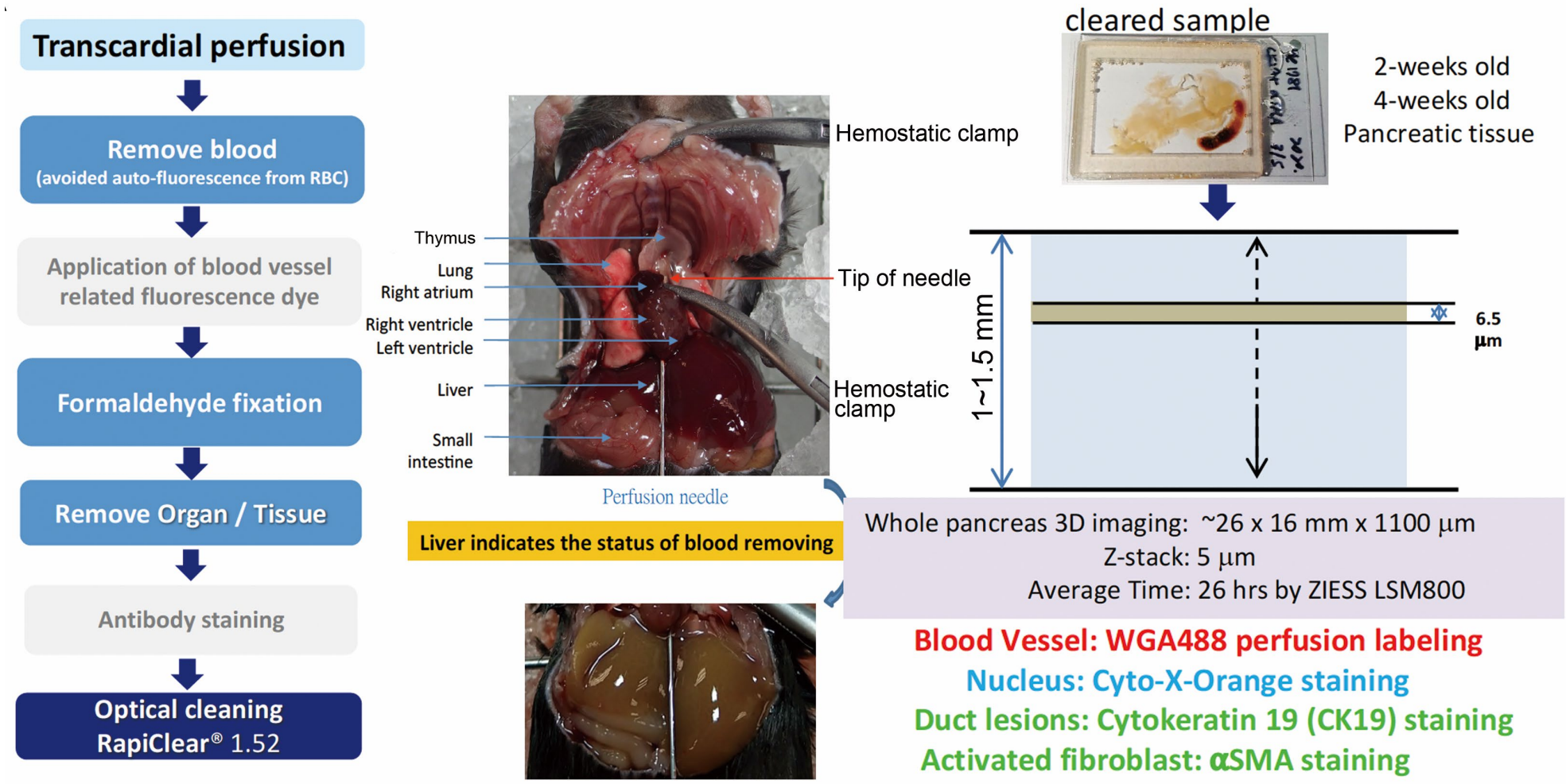
Genetic alterations
Dysregulation of Genes expression

pancreatic microenvironment

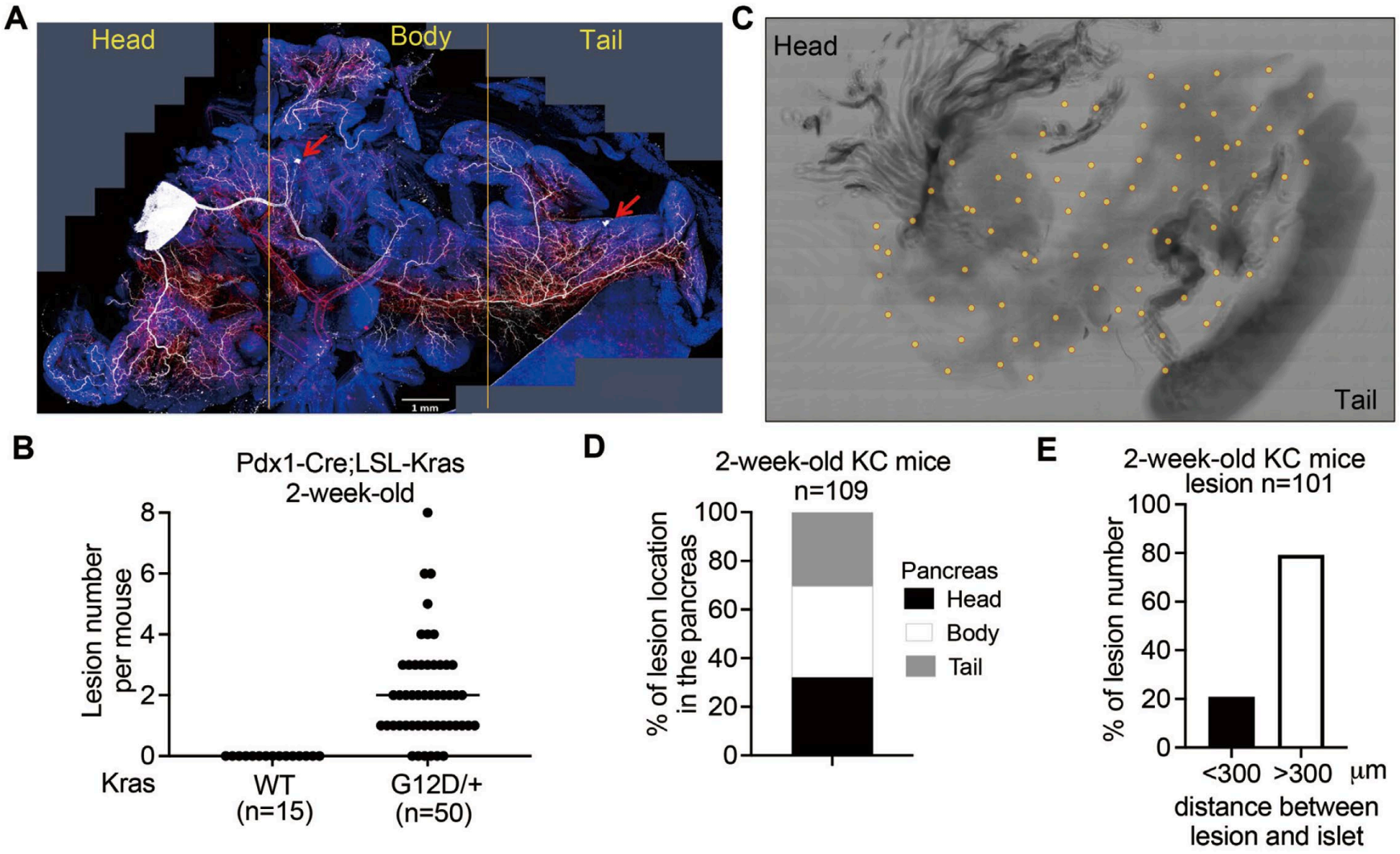
Growth advantage and PanIN initiation

Material: 2- and 4- week-old Pdx1-Cre;LSL-KRas$^{G12D/+}$ mice (KC)
2- and 4- week old Pdx1-Cre;LSL-Kras$^{+/+}$ mice (control)

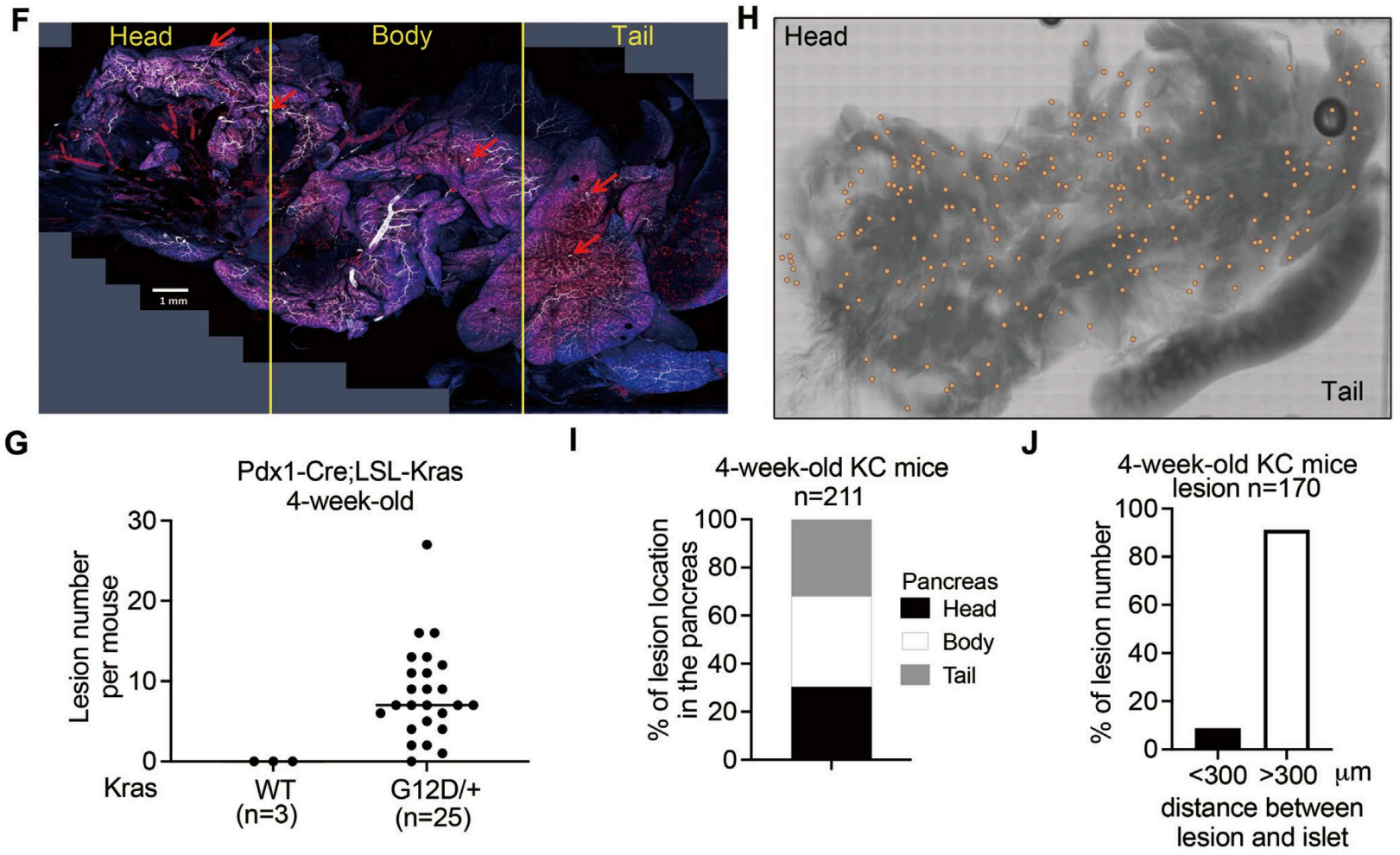
Method: Whole pancreas 3D histology



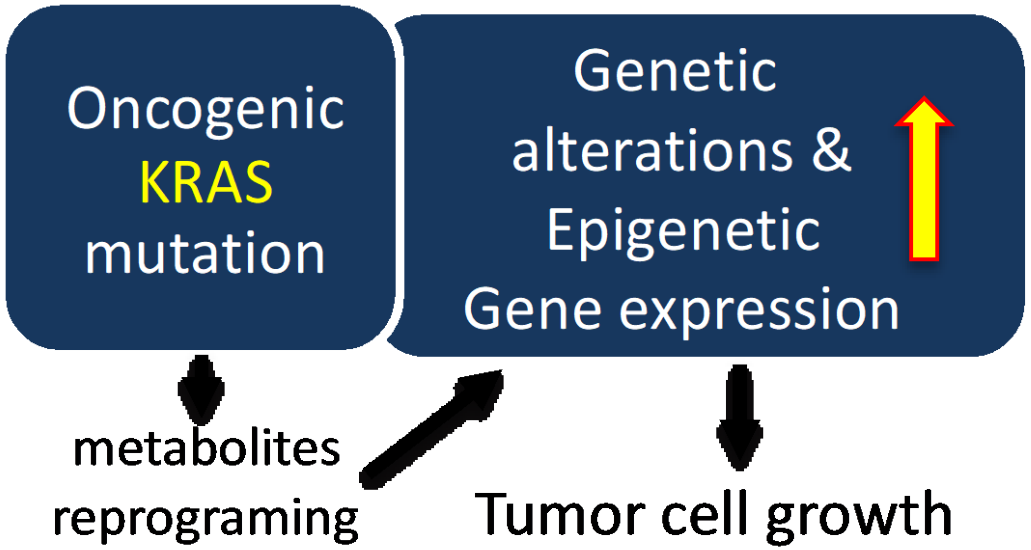
在兩週大Kras^{G12D}突變小鼠胰臟可以偵測到最早的胰臟癌前病變組織,胰臟上皮內瘤(PanIN)是隨機分佈在胰臟中的



四週大Kras^{G12D}突變小鼠胰臟也可以偵測到早期的PanIN，數目增加，但也是隨機分佈在胰臟中



PanIN啟動過程中，內在基因改變可能先於微環境影響



Previous studies

hypothesis

$Kras^{G12D}$

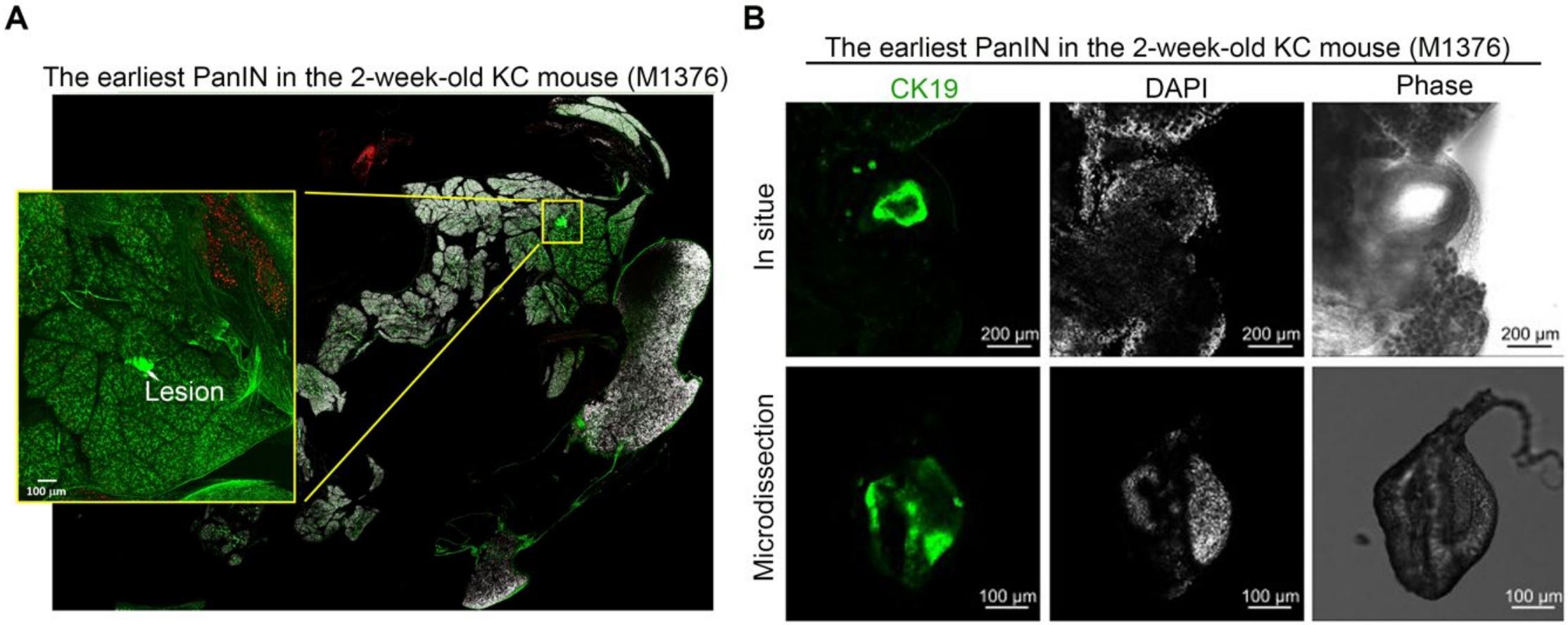
Genetic/Epigenetic regulation

Key genes activation

$Kras^{G12D}$ + other activated genes

PanIN initiation

分析最早PanIN的基因突變和基因表現差異



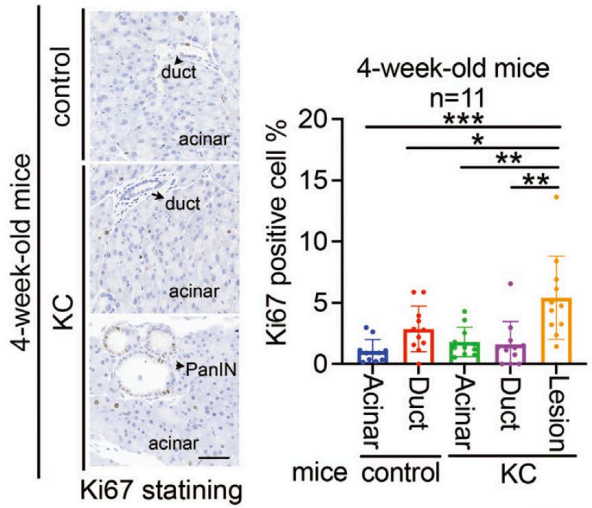
MUC4 過度表現可能在 PanIN 的啟動中發揮關鍵作用

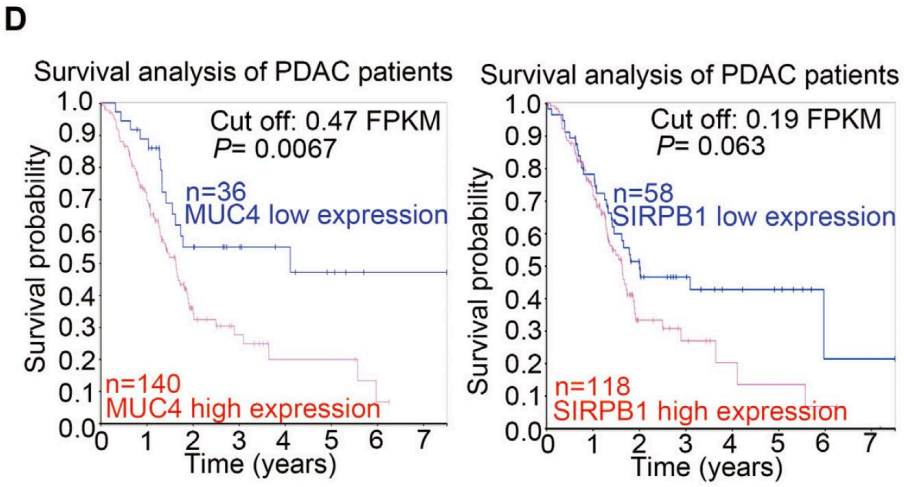
A

Gene Name	Gene ID	Chromosome	Region	Type	Reference	Allele	Type	Amino acid change	Number of Samples (Only Lesion)	Percentage of Samples (Lesion)
Muc4	140474	chr16	32752343	SNV	T	G	Exon	-	7	33.33%
Muc4	140474	chr16	32752384	SNV	C	G	Exon	NP_536705.3:p.Ala754Gly	7	33.33%
Muc4	140474	chr16	32752385	SNV	T	A	Exon	-	7	33.33%
Muc4	140474	chr16	32752398	SNV	C	A	Exon	NP_536705.3:p.His759Asn	7	33.33%
Muc4	140474	chr16	32755752^32755753	Insertion	-	GG	Exon	NP_536705.3:p.Asn1877fs	8	38.10%
Muc4	140474	chr16	32755754	Deletion	T	-	Exon	NP_536705.3:p.Asn1877fs	8	38.10%
Muc4	140474	chr16	32755757	Deletion	T	-	Exon	NP_536705.3:p.Ser1878fs	8	38.10%
Muc4	140474	chr16	32755843	SNV	T	G	Exon	NP_536705.3:p.Phe1907Cys	7	33.33%
Muc4	140474	chr16	32755857	SNV	G	C	Exon	NP_536705.3:p.Val1912Leu	7	33.33%
Sirpb1a	320832	chr3	15416972	SNV	T	G	Exon	NP_001002898.1:p.[Met99Leu]	8	38.10%
Sirpb1a	320832	chr3	15417001	SNV	T	C	Exon	NP_001002898.1:p.Asn89Ser	7	33.33%

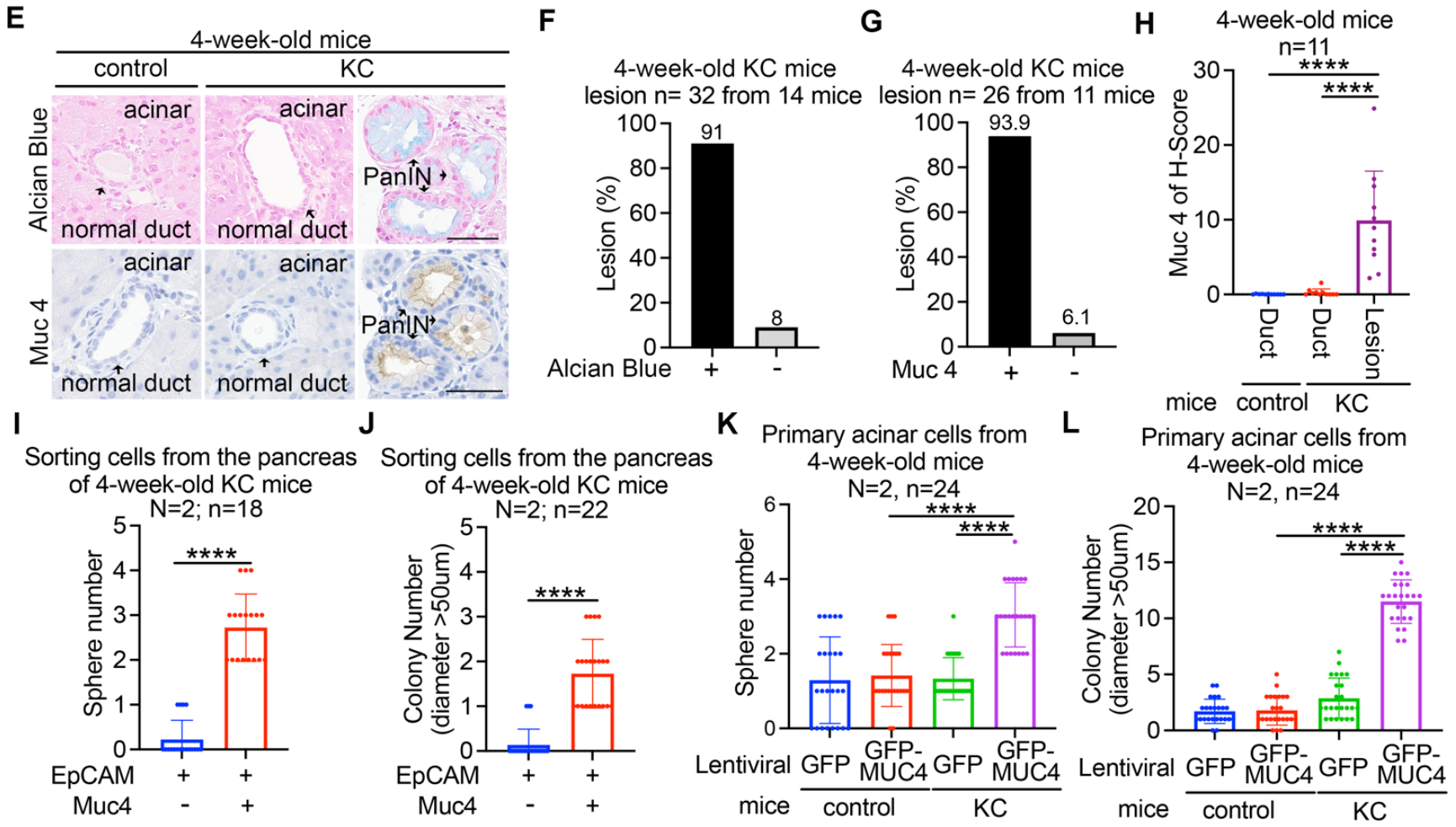
B

Gene Name	Gene ID	Chromosome	Gene length	Exons	Fold Change - M1376-Lesion / M1376-Ctrl	RPKM-M1376-Ctrl	RPKM - M1376-Lesion	Total exon reads - M1376-Ctrl	Total exon reads - M1376-Lesion
Muc4	140474	chr16	46506	25	2.0778	0.3356	0.6972	170	372
Sirpb1a	320832	chr3	54800	13	1.7408	0.1471	0.2561	12	22



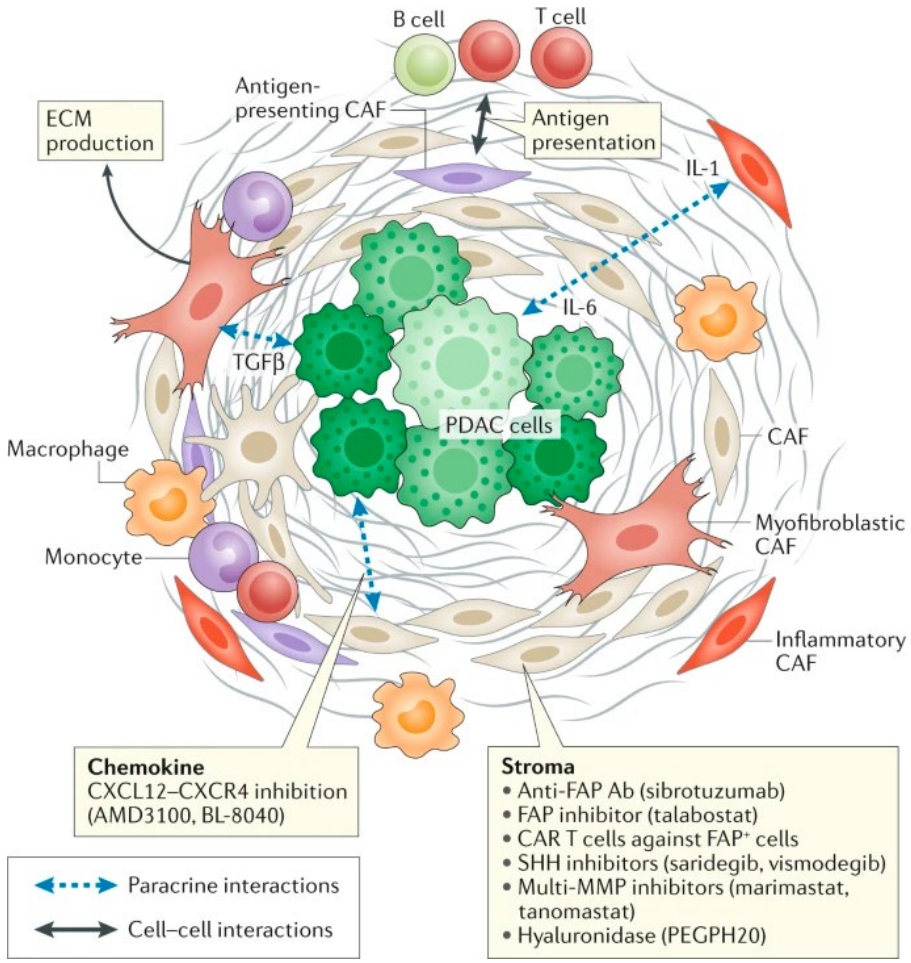


同時表現致癌Kras^{G12D}基因和MUC4基因可以驅動胰臟細胞癌



什麼樣的微環境細胞參與促進早期 PanIN 的形成?

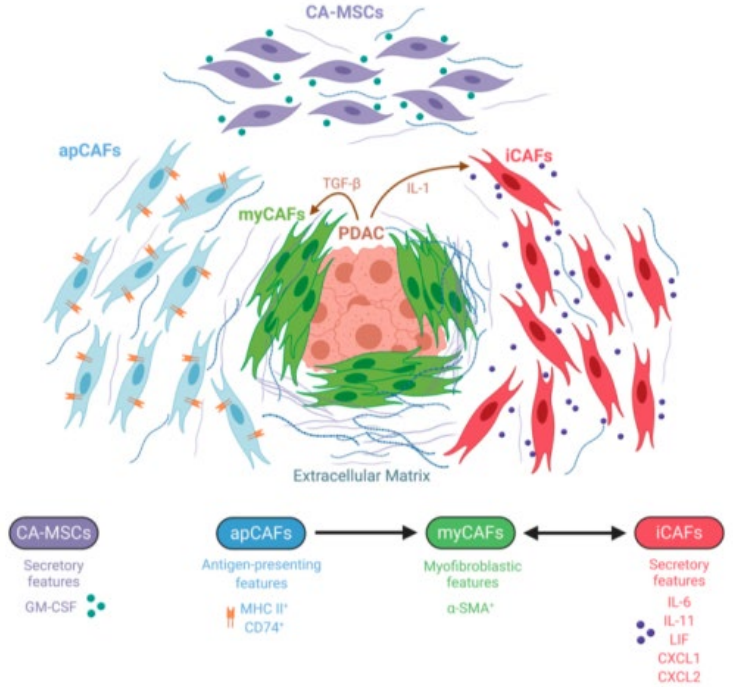
Pancreatic tumor microenvironment (TME)



Nature Reviews Clinical Oncology volume 17, pages527–540(2020)

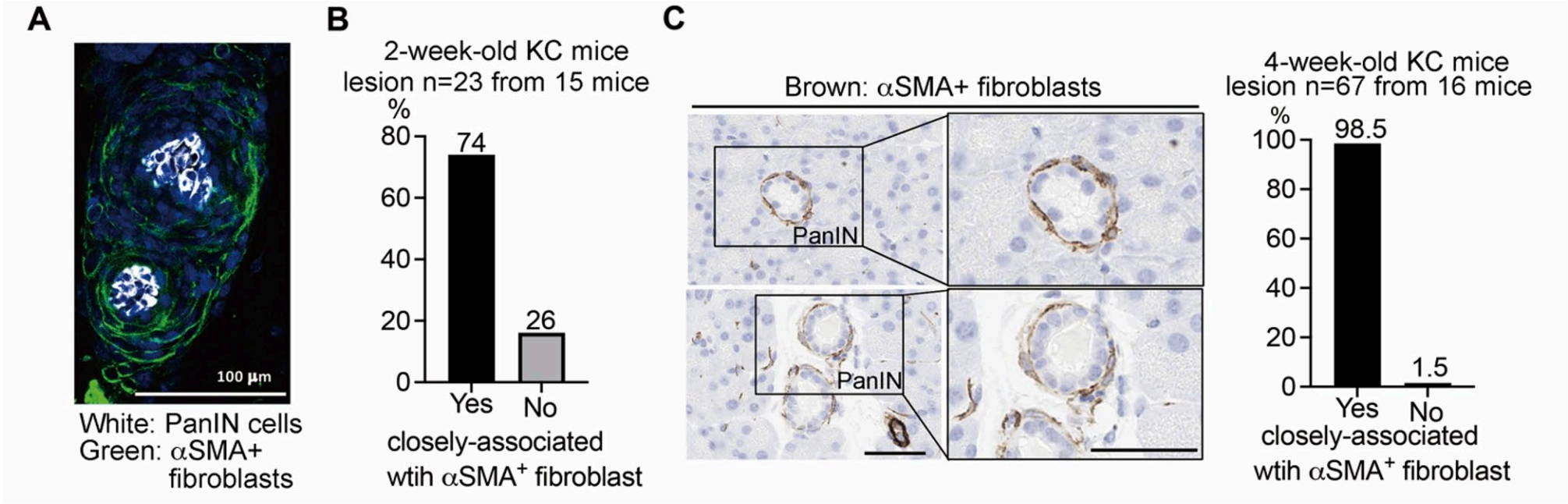
Cancer-associated fibroblasts (CAFs) are the most abundant stromal cells (up to 80% of the tumor mass in pancreatic tumors) contributing to a desmoplastic stroma in PDAC

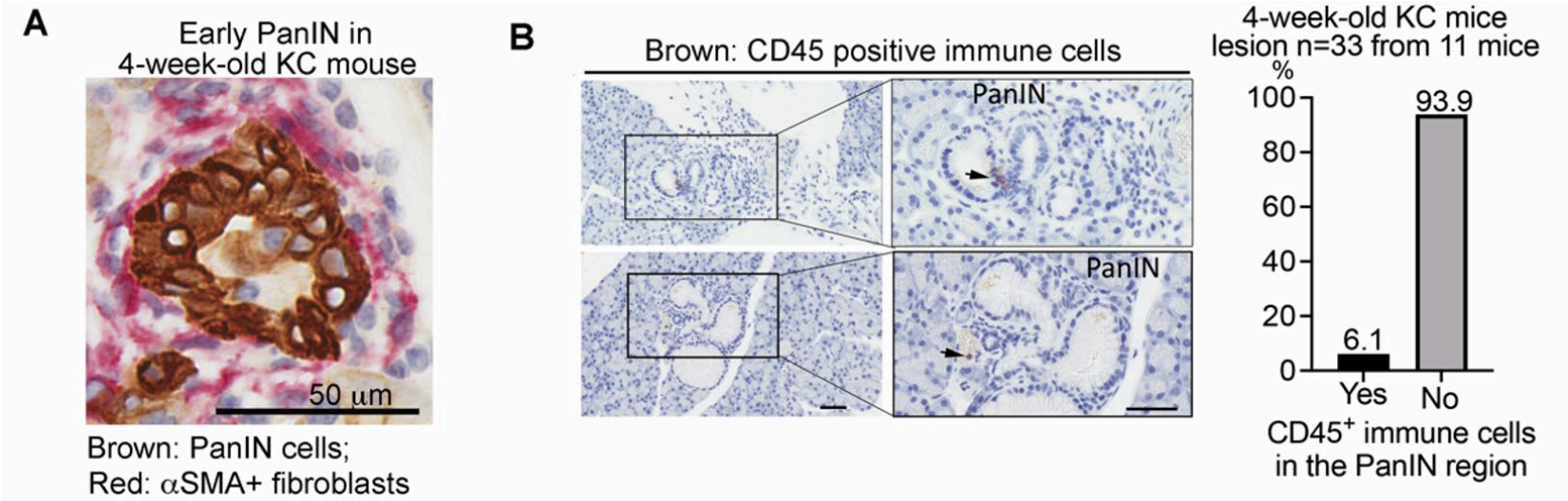
Yu, M. & Tannock, I. F.
Cancer Cell **21**, 327-329 (2012).



Int J Mol Sci. 2020 Jul 31;21(15):5486.

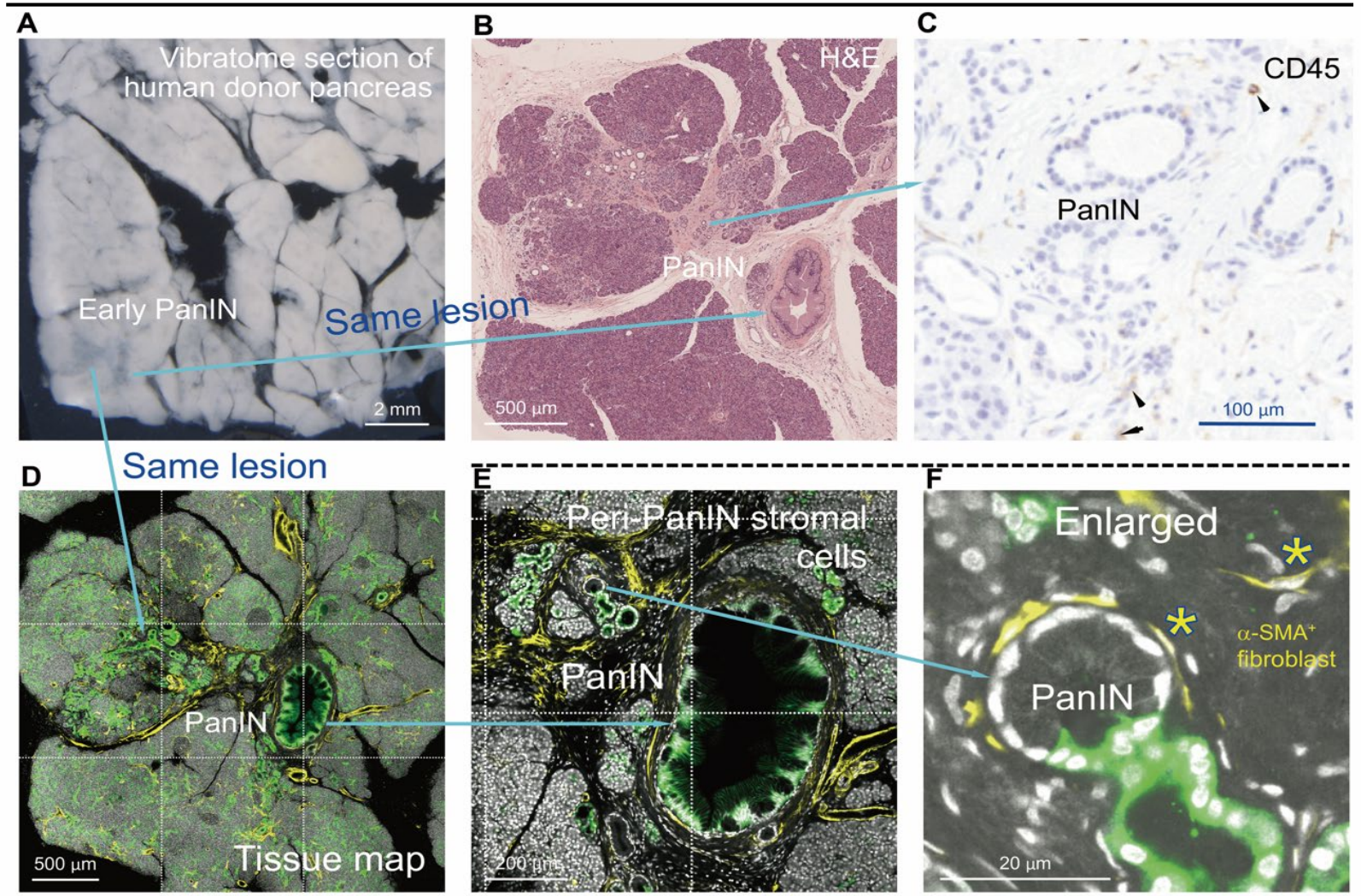
表現αSMA的 纖維母細胞而非免疫細胞與早期 PanIN 細胞密切相關





在人類胰臟檢體中也可以發現早期PanIN與表現αSMA的纖維母細胞緊密相連

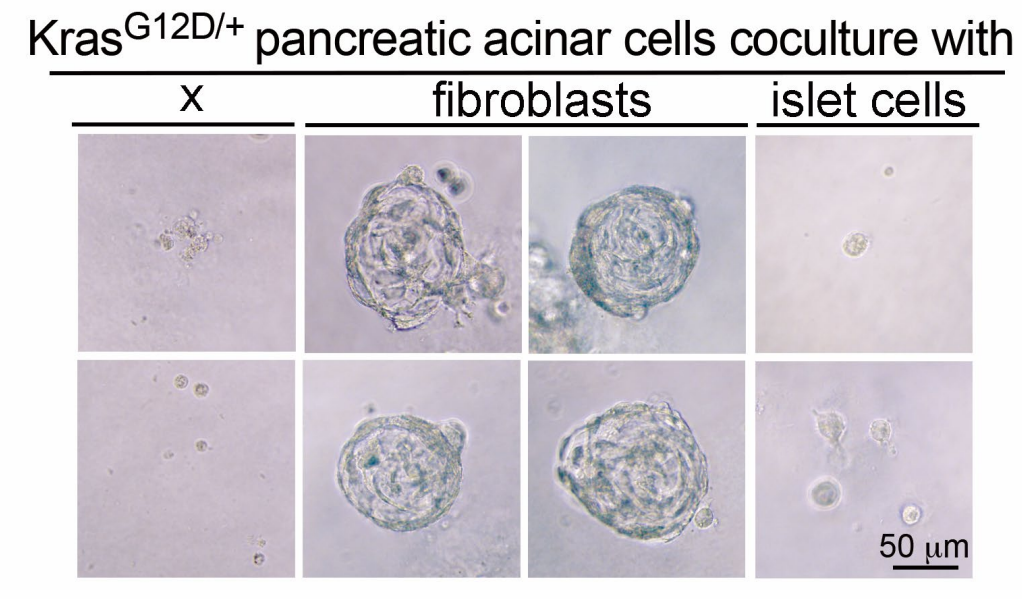
Human pancreatic specimen with early PanINs

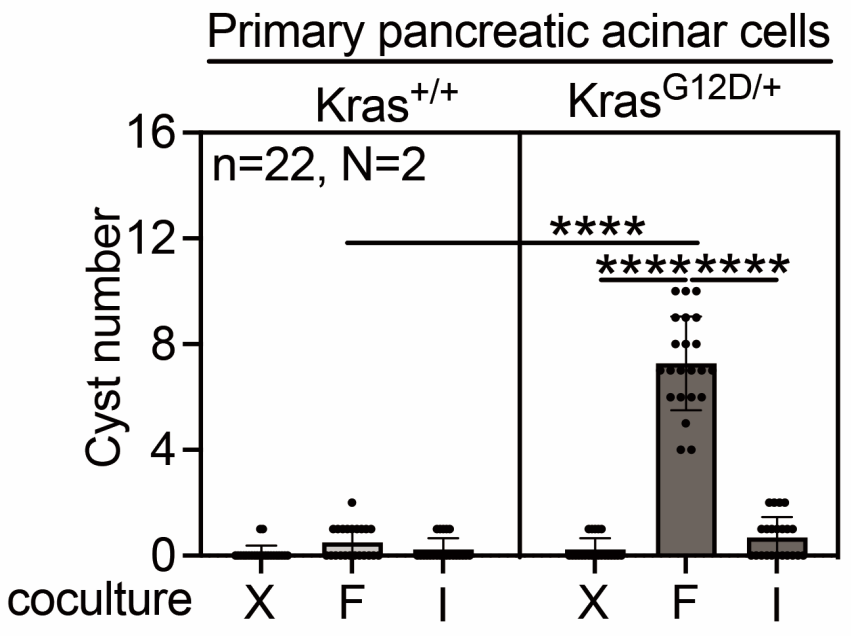


與 PanIN 細胞密切相關的成纖維細胞的生物學作用是什麼?

纖維母細胞是否可以促進PanIN的形成?

$Kras^{G12D}$突變的胰臟細胞與纖維母細胞共同培養可以促進PanIN形成





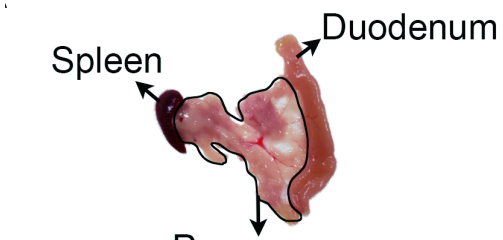
X: Pancreatic cells only (1000 cells)
F: coculture with pancreatic fibroblast (2000 cells) from $Kras^{+/+}$ mice
I: coculture with islet cells (2000 cells) from $Kras^{+/+}$ mice

In the pancreas, acinar cells make up about 85% of volume and ducts are 5%.

Acinar-to-Ductal Metaplasia (ADM) is considered to be one of the main sources of PanIN.

Only duct/duct-like cells (ADM, PanIN) can form cyst.

$Kras^{G12D}$突變的管狀細胞與纖維母細胞緊密相連容易促進癌化



Cut to 1 mm^3

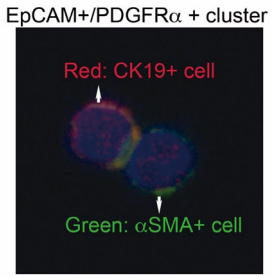
Mechanical dissociation

40 μm filter

EpCAM Ab/ PDGFRα Ab staining

FACS analysis

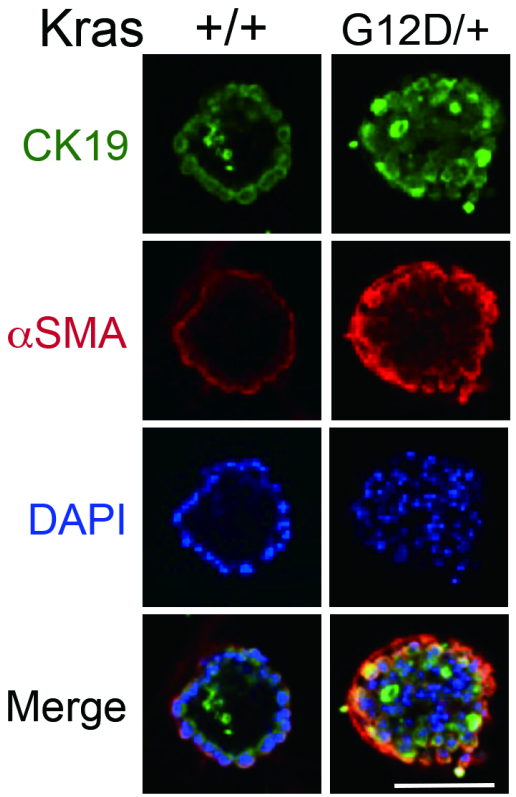
Sorting EpCAM+/PDGFRα+ cluste



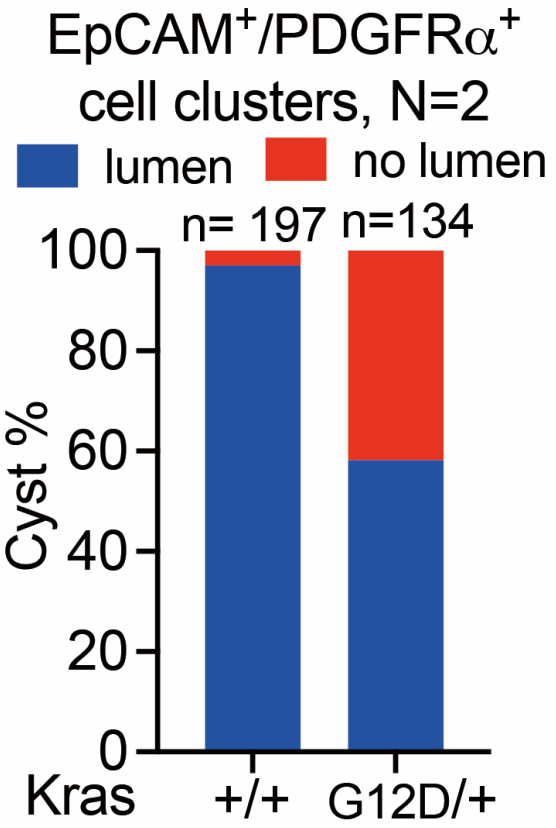
Up: Cells in 2% matril gel (200 μl)
Down: Coating 50% matrigel (200 μl)

Extraction of Cyst

Immunofluorescence staining with CK19 Ab and αSMA Ab



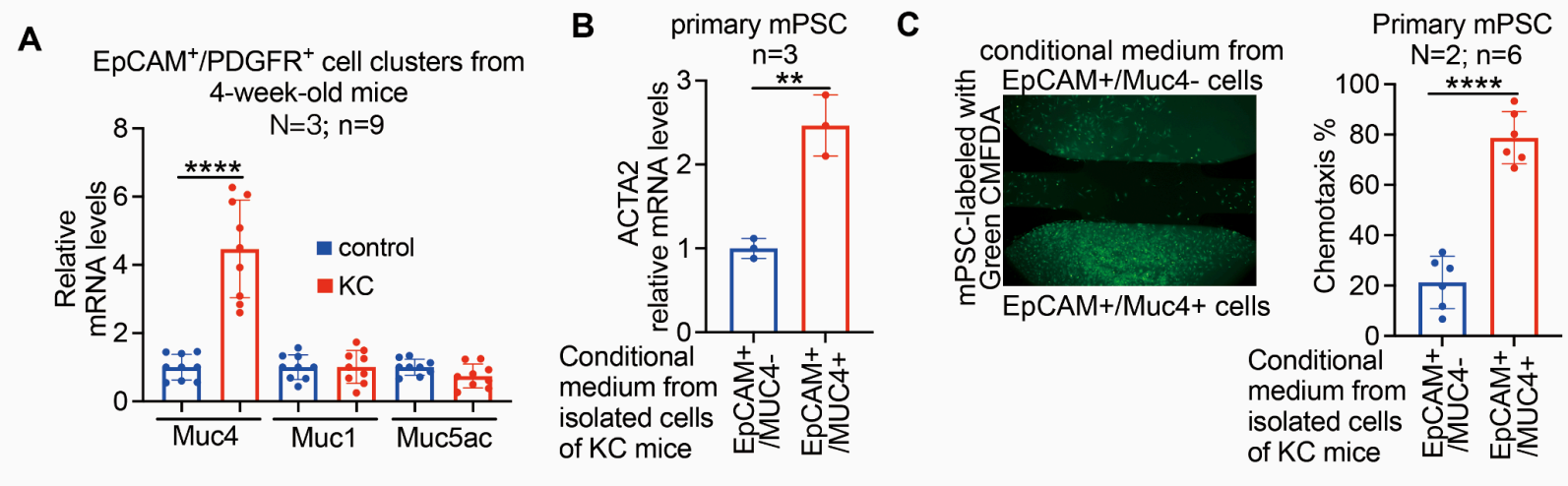
Cysts devoid of a lumen, underscoring their inherent potential for cellular transformation

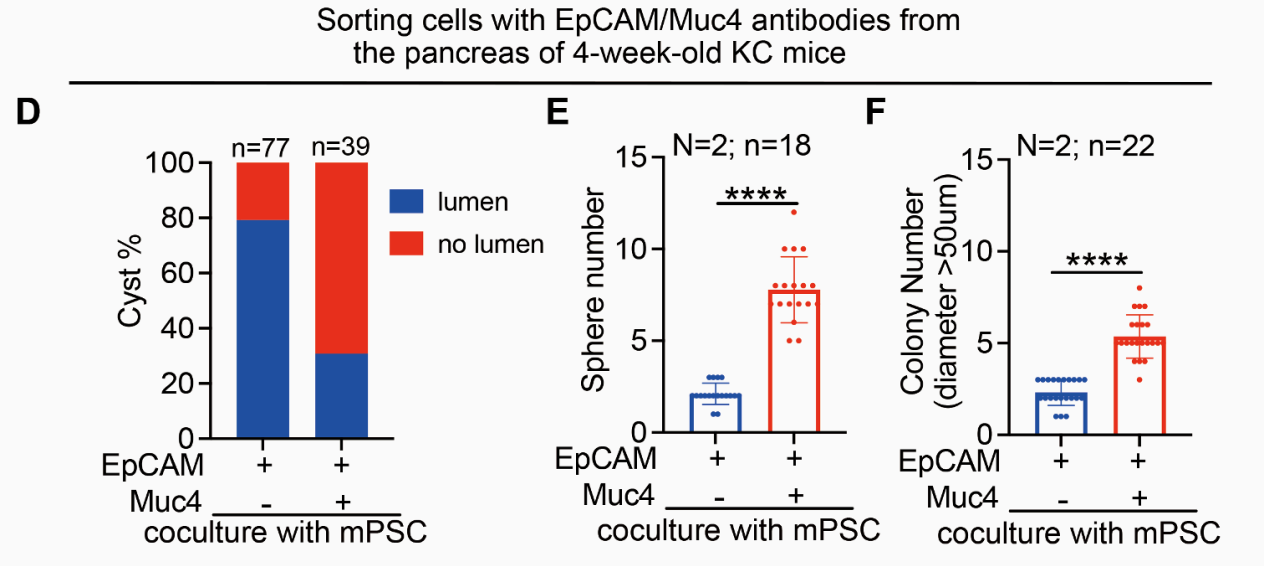


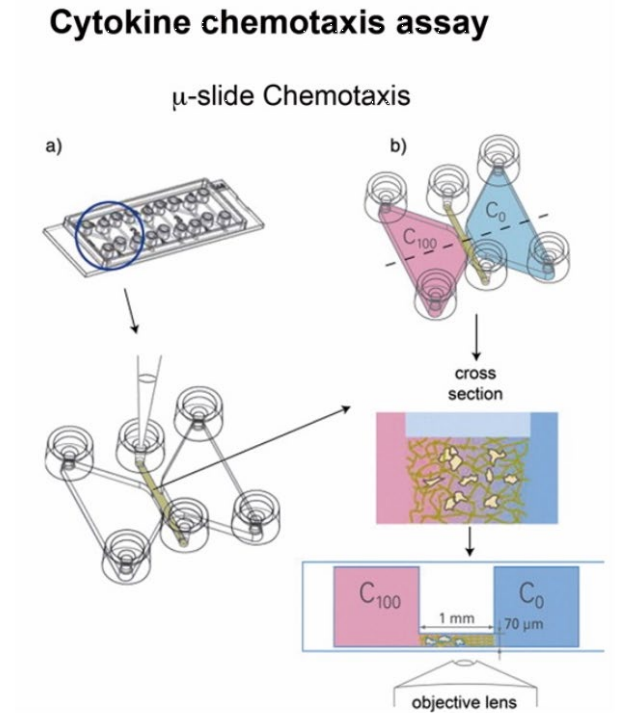
綜上所述，這些數據表明 Muc4 過度表達和緊密相連的纖維母細胞有利於 $Kras^{G12D}$ 突變胰臟細胞形成 PanIN。

PanIN 中 Muc4 過度表現與纖維母細胞關聯之間的潛在交互作用為何?

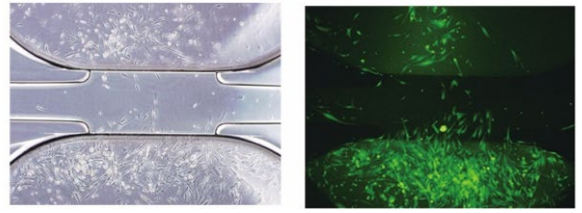
致癌Kras^{G12D}基因和Muc4基因過度表達的胰臟細胞容易活化和吸引纖維母細胞



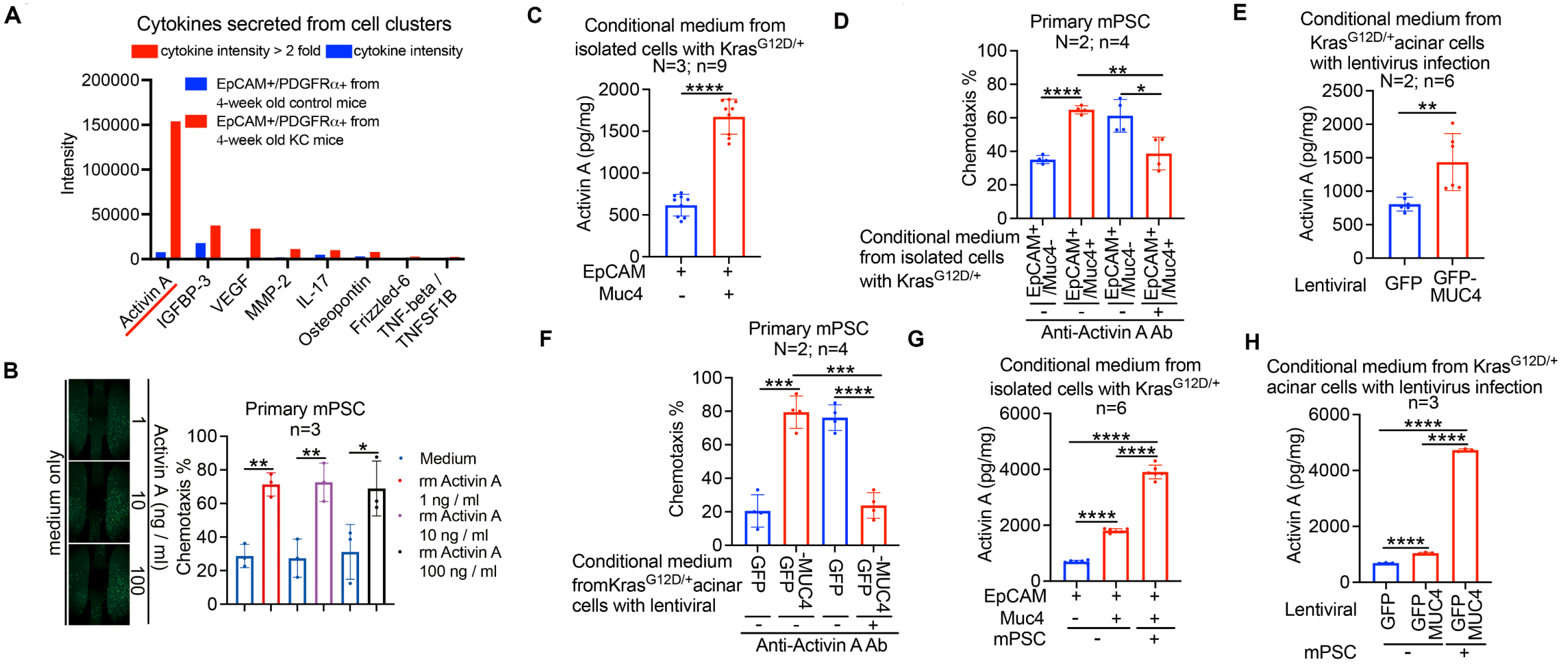




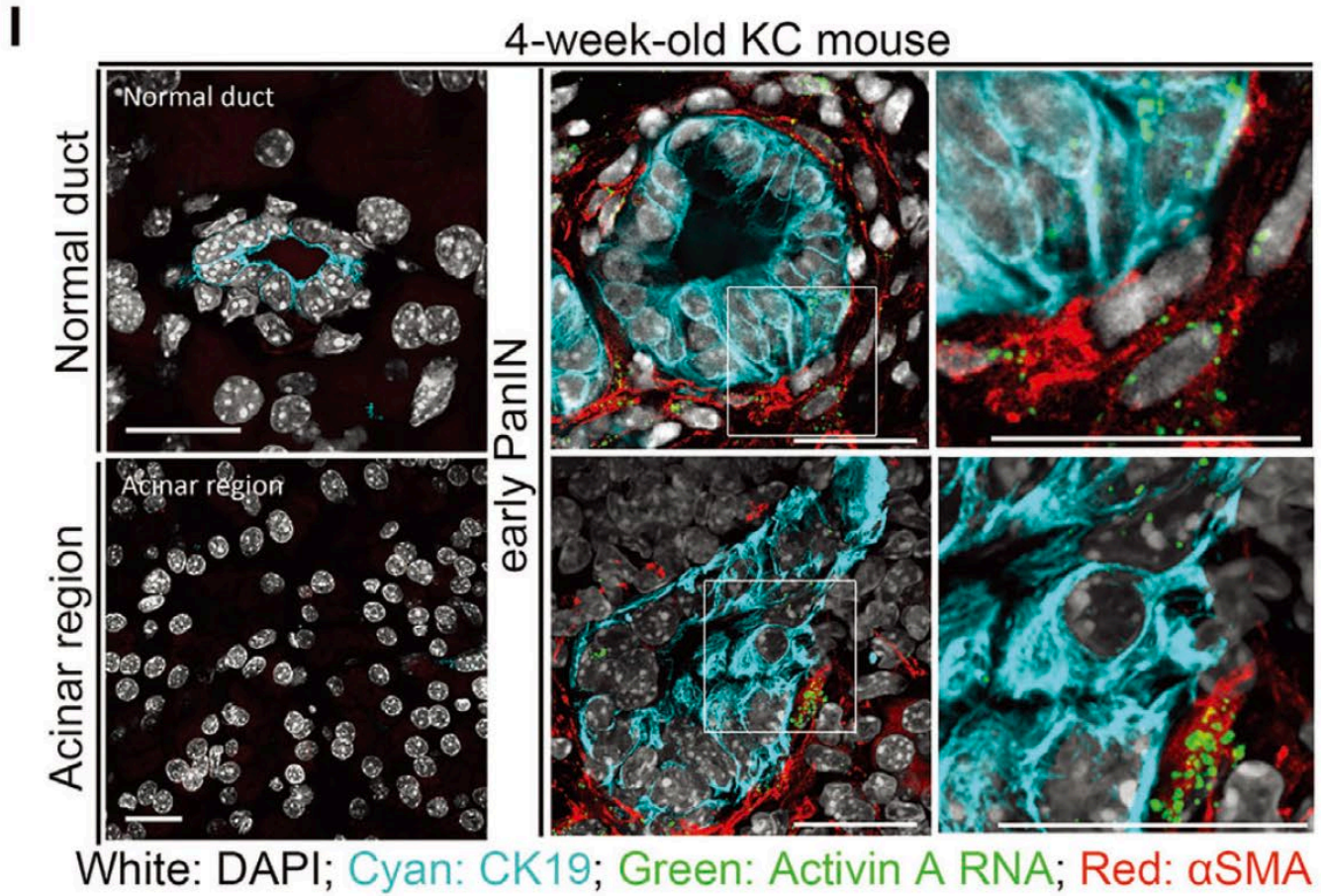
Cell stained with Cell Tracker Green CMFDA

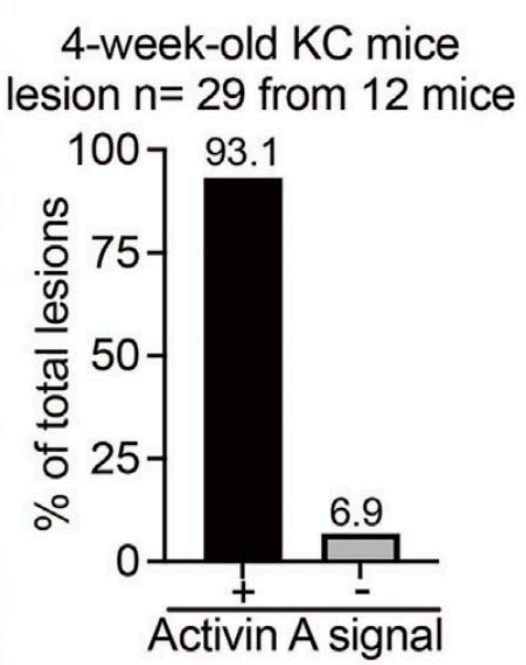


致癌Kras^{G12D}基因和Muc4基因過度表達的胰臟細胞會釋放小量Activin A吸引纖維母細胞，進而促進纖維母細胞產生更多Activin

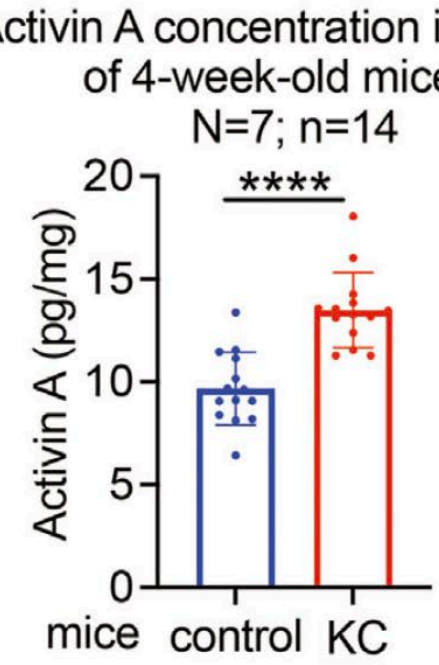


在4週大Kras^{G12D}突變小鼠中，大部分早期PanIN細胞都表現Activin A mRNA



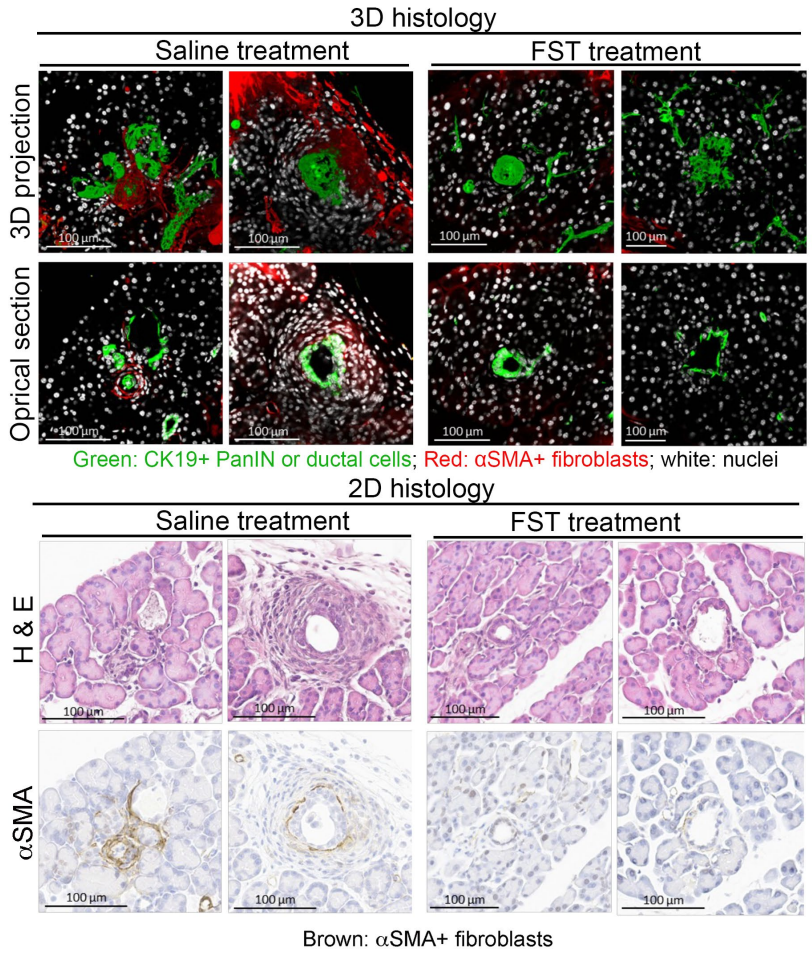


J

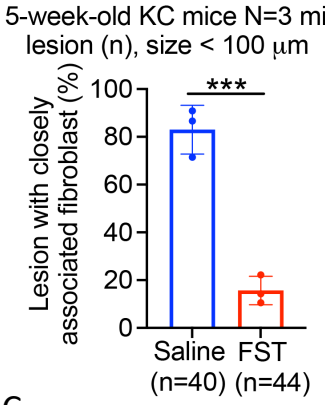


使用卵泡抑素(Follistatin,FST) 阻斷 Activin A 訊號傳導可以減少早期 PanIN 中纖維母細胞的募集，從而抑制 $Kras^{G12D}$突變小鼠中 PanIN 的形成和生長

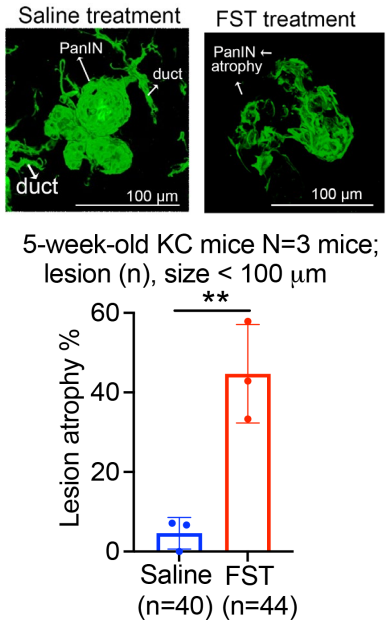
A



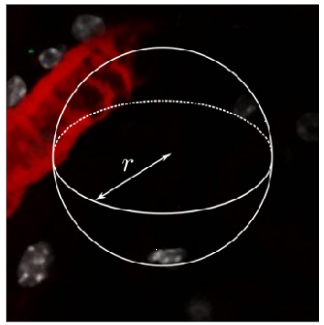
B



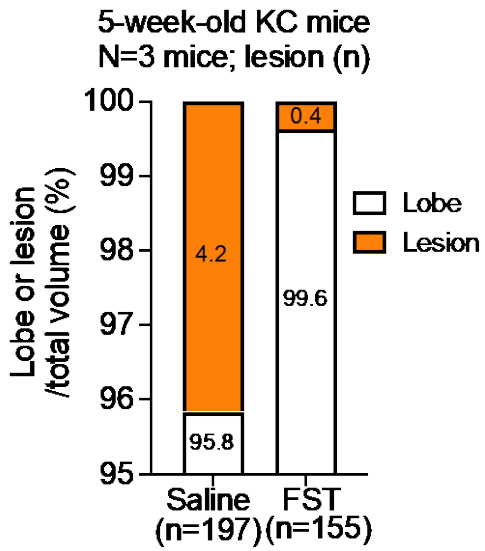
C



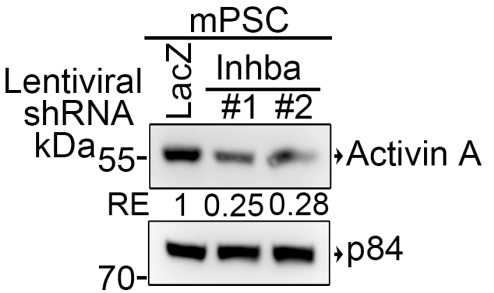
D



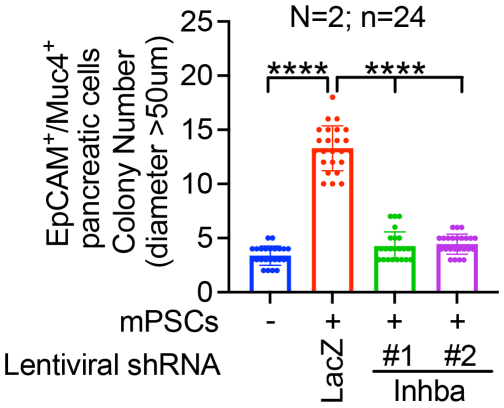
Assuming lesion is a sphere, the formula for the volume of a sphere is $V = 4/3\ \pi r^3$ where V = volume and r = radius



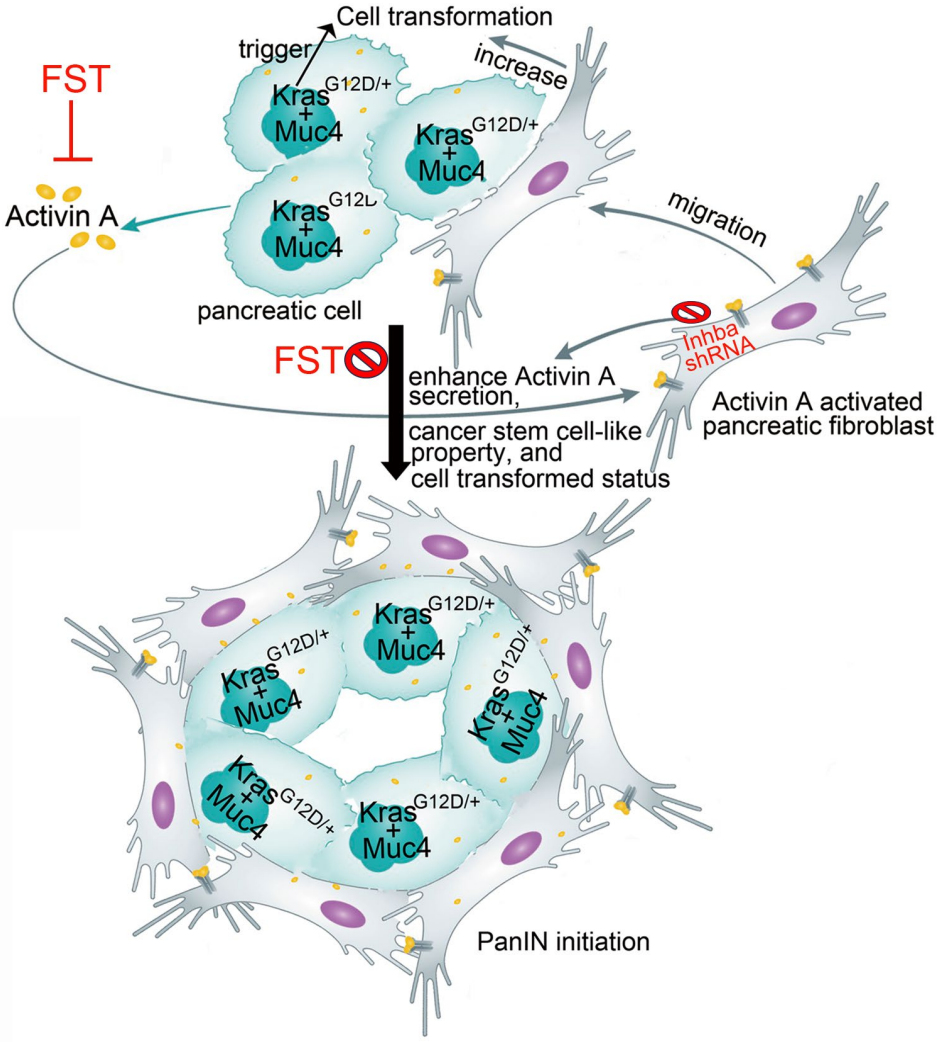
E



Coculture $EpCAM^+/Muc4^+$ pancreatic cells and mPSCs with indicated lentiviral shRNA



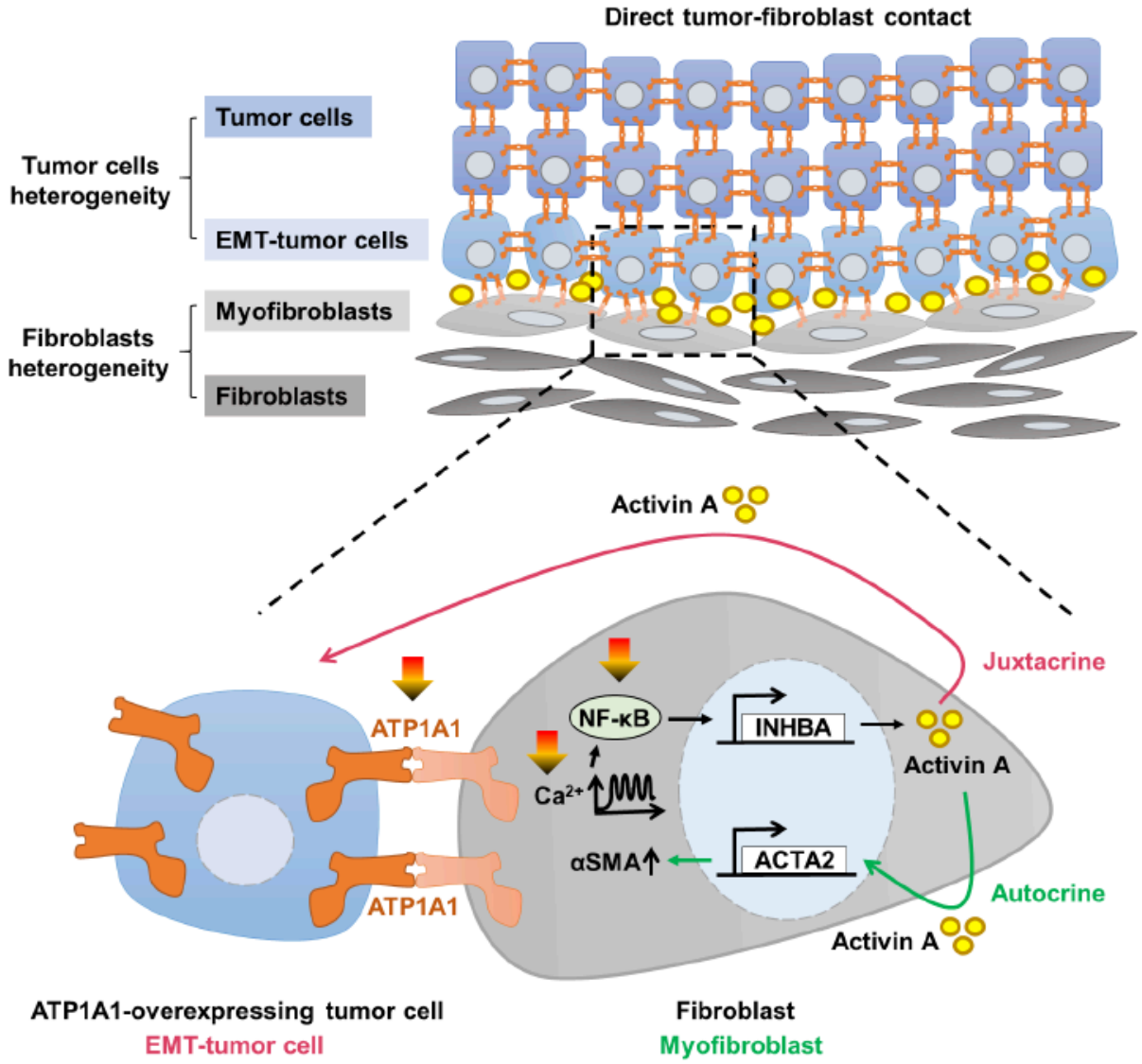
揭開致癌Kras^{G12D}基因驅動胰臟癌前病變的神秘面紗



Hu et al. Adv. Sci. 2023; 2301240:1-20

當Kras$^{G12D/+}$和Muc4基因一同發揮作用時，它們會促進細胞生長並釋放少量的Activin A。這會啟動並吸引纖維母細胞，進而刺激它們釋放更多的Activin A，進一步導致胰臟細胞的癌變和PanIN（胰臟上皮內瘤）的形成。

細胞直接接觸是引發胰臟癌轉移的關鍵



ATP1A1過度表現的癌細胞，與周圍纖維母細胞以ATP1A1連結，並誘導纖維母細胞分泌Activin A，增加癌細胞上皮-間質轉化(EMT)的特性及纖維母細胞活化，促進腫瘤侵犯及轉移。

Chen, et al. Nature Communications. 2022 May 26. 13: 2945.