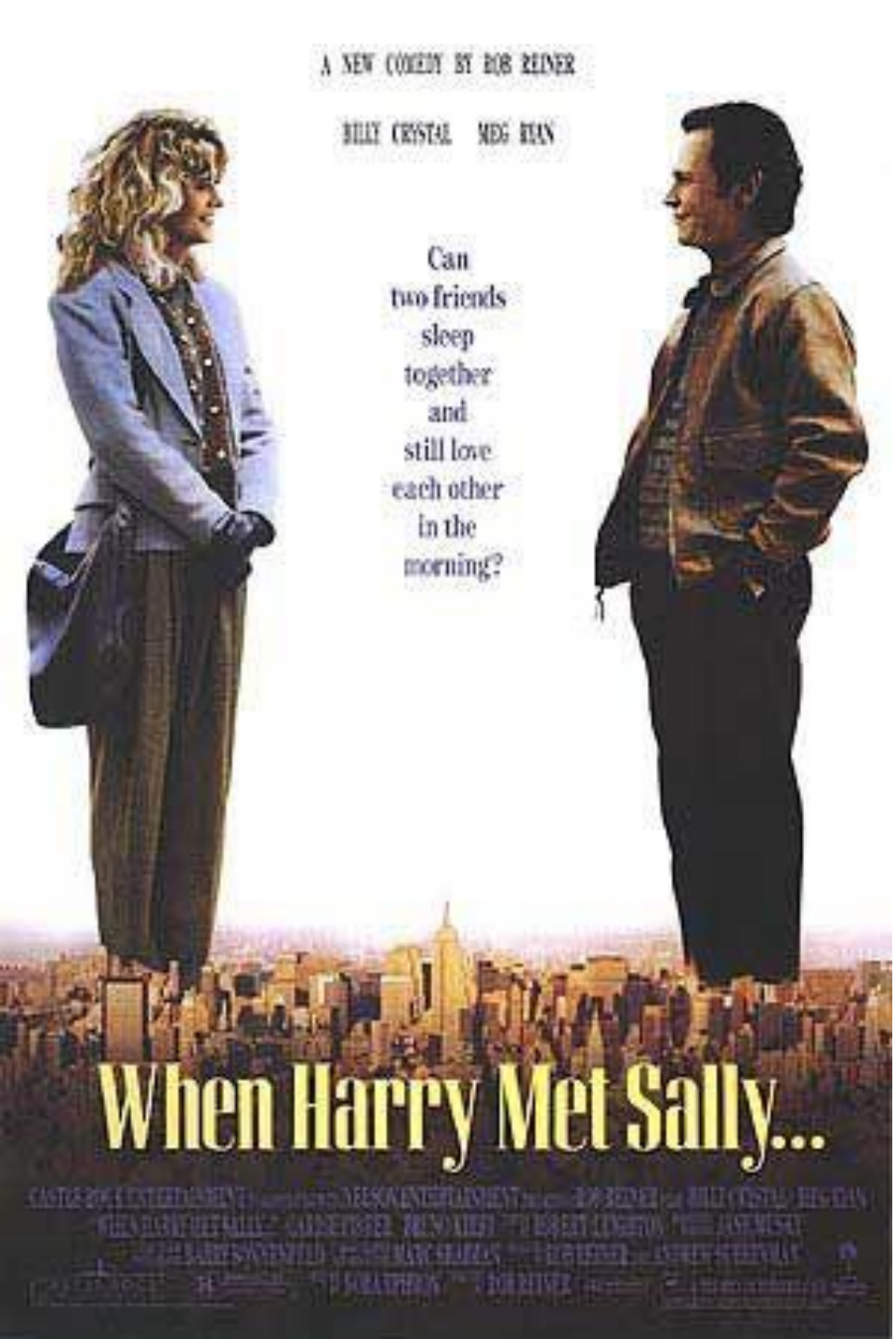
以蛋白質體學發展癌症治療新策略

高雄醫學大學
醫學影像暨放射科學系
田育彰



~當哈利遇見莎莉~(1989)

哈利與莎莉這兩個人的的確確就是一對命中註定的戀人，唯一的問題是他們自己並不知道。所以從敘事的觀點來看，他們不知道彼此應當相愛，就是阻礙他們相愛的原因，如何克服這個障礙就是故事推展的動力所在。

影片討論 李振亞

以研究而言，不知道不同領域應當相結合，就是阻礙整合科學的原因。

What Is Proteome?

Definitions of Proteomics

- First coined in 1995 by Wilkins
- Be defined as the large-scale characterization of the entire protein complement of a cell line, tissue, or organism.
- The study of proteomes
- Goal:
  - To obtain a more global and integrated view of biology by studying all the proteins of a cell rather than each one individually

Nobel Prize in Chemistry 2002

"for the development of methods for identification and structure analyses of biological macromolecules"

"for their development of soft desorption ionisation methods for mass spectrometric analyses of biological macromolecules"

NMR

John B. Fenn

ESI

b. 1917

Koichi Tanaka

MALDI

b. 1959

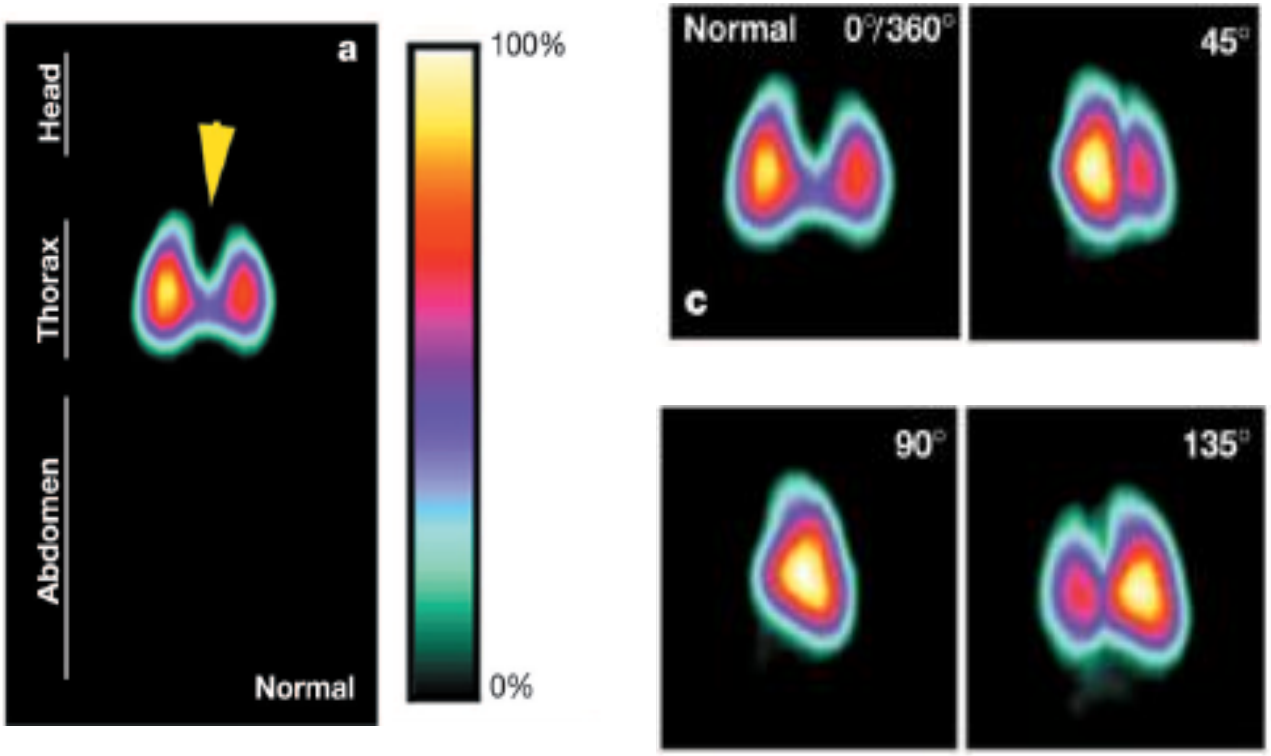
Subtractive proteomic mapping of the endothelial surface in lung and solid tumours for tissue-specific therapy

NATURE, VOL 429, 2004: 629-635

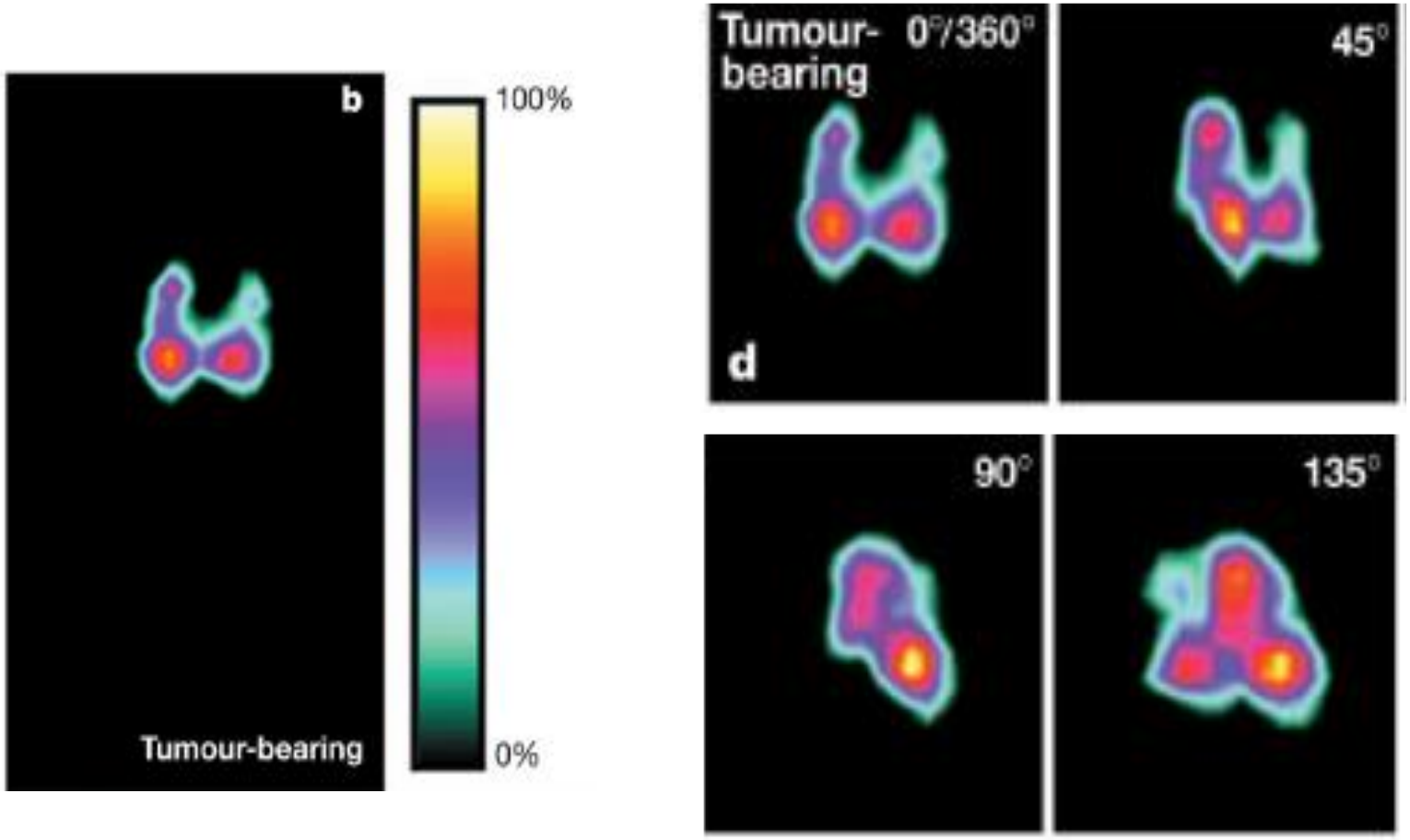
Lung-specific targeting in vivo

- Aminopeptide P (APP)
- intravenously injected ^{125}I-labelled monoclonal antibodies into rats and performed wholebody imaging using planar γ-scintigraphy.

High resolution single photon emission computed tomography (SPECT) imaging

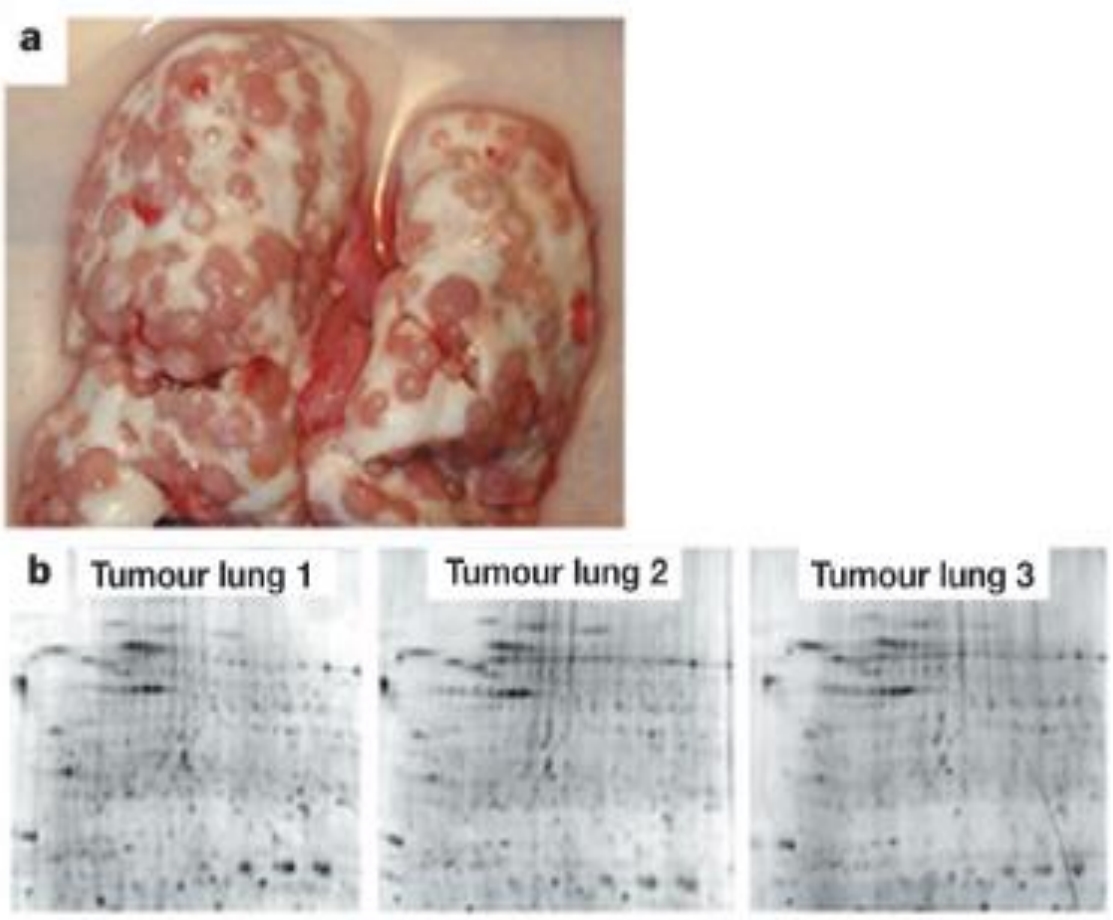


APP expression in the rat appeared quite specific for normal lung tissue



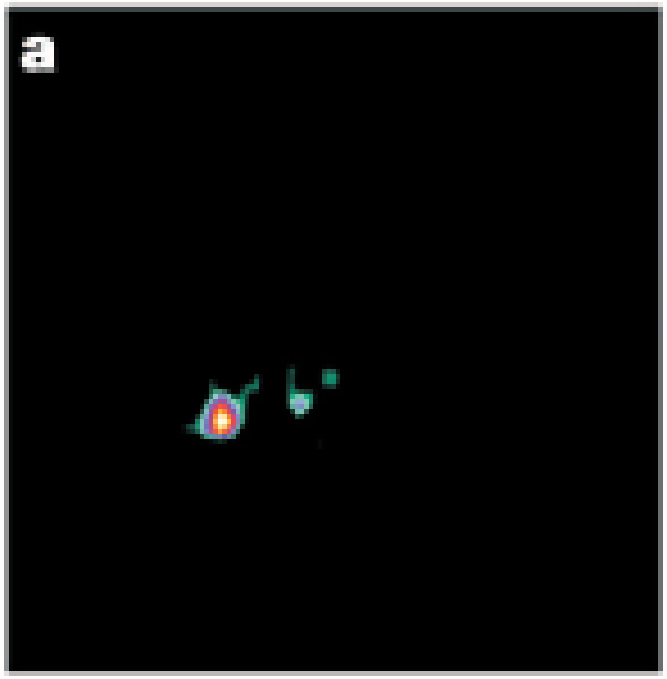
APP was reported as an expressed homing peptide on mouse blood vessels of breast and mammary adenocarcinomas

Tumour-induced endothelial cell proteins

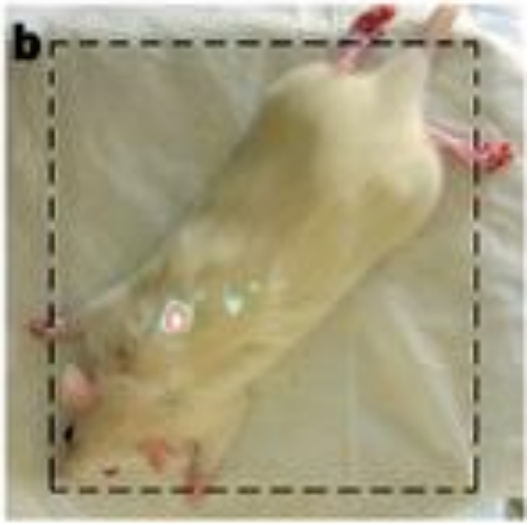


2D gels in endothelial cell plasma membranes from normal lungs versus tumours in lungs.

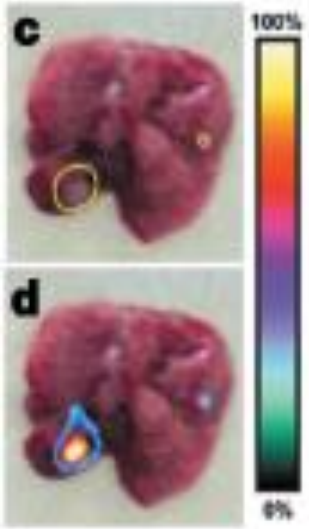
Targeting and imaging of solid tumours



(A). Whole-body planarg scintigraphic imaging 4 h after injection of ^{125}I-AnnA1 antibodies



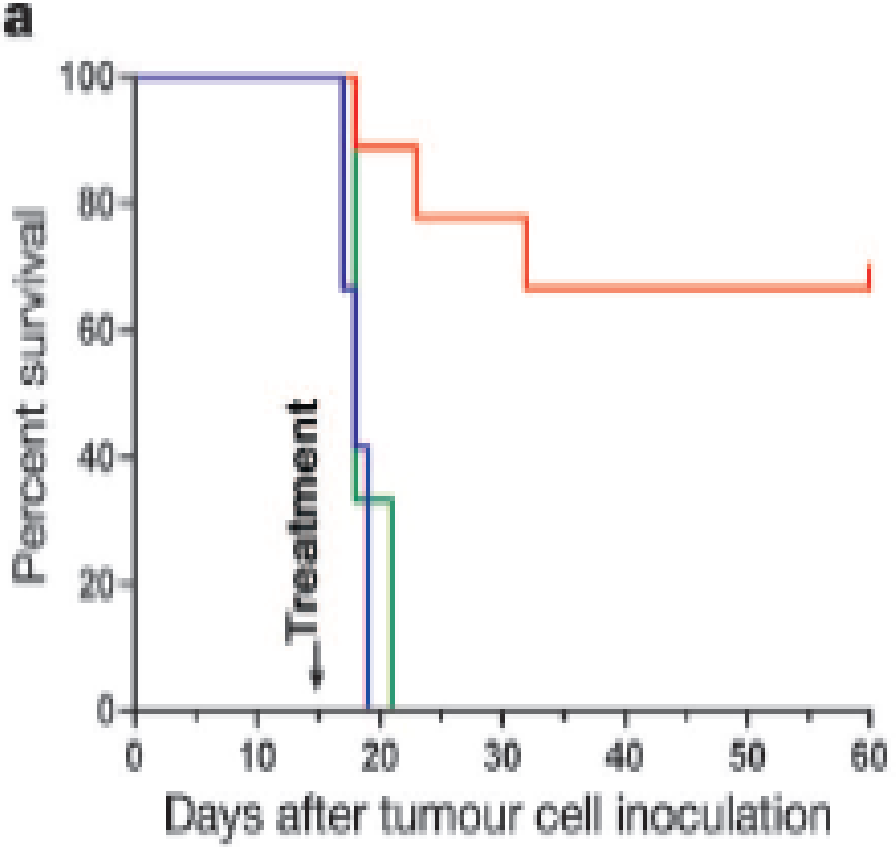
(B). ^{125}I-AnnA1 antibody signal superimposed onto photo of experimental animal lying on the detector plate.



(C). Digital image of excised lungs showing location of tumours, circled in yellow

(D). Overlay of planar images of tumour hot spots with excised tumour-bearing lungs

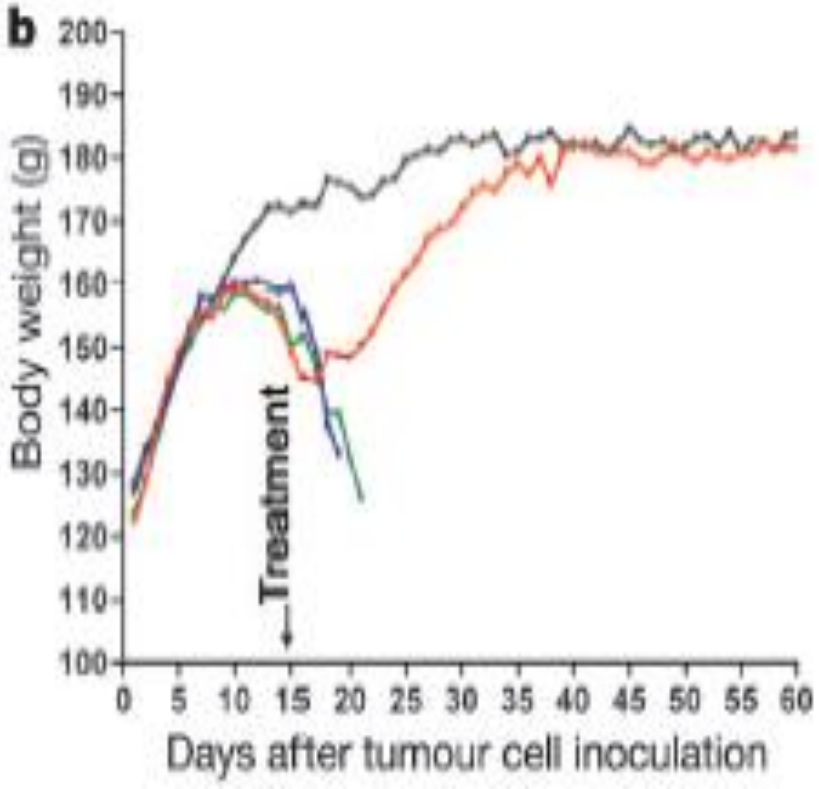
Radio-immunotherapy of solid tumours



Red line: tumor bearing rats treated with ^{125}I-AnnA1 antibody

Green line: untreated tumor bearing rats

Blue line: tumor bearing rats treated with control ^{125}I-IgG



Red line: tumor bearing rats treated with ^{125}I-AnnA1 antibody

Green line: untreated tumor bearing rats

Blue line: tumor bearing rats treated with control ^{125}I-IgG

Black line: normal rats

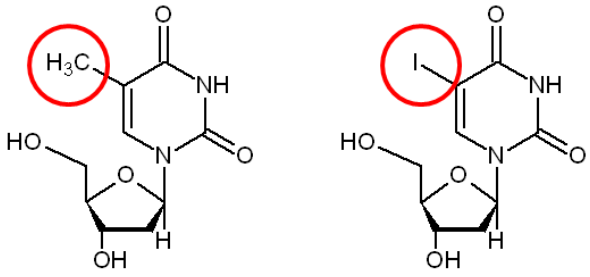
Utilizing proteomic analysis to study the efficacy and mechanisms of 5-iodo-2-deoxyuridine target therapy of breast cancer in mice

- Iododeoxyuridine (IUdR)為形成DNA物質thymidine 的類似物，可在細胞行有絲分裂(S期)時被吸收成為其DNA的一部份。惡性腫瘤細胞生長快速，其DNA的複製也較一般細胞快，因此動物實驗注射放射性碘標幟的IUdR 被腫瘤細胞吸收後，一方面可藉著放射性的示蹤性質精確掌握惡性腫瘤的位置，另一方面可利用放射性IUdR釋出的放射線殺死腫瘤細胞，達到分子造影診斷與治療的雙重目的。

Radiosensitizer – Iododeoxyuridine

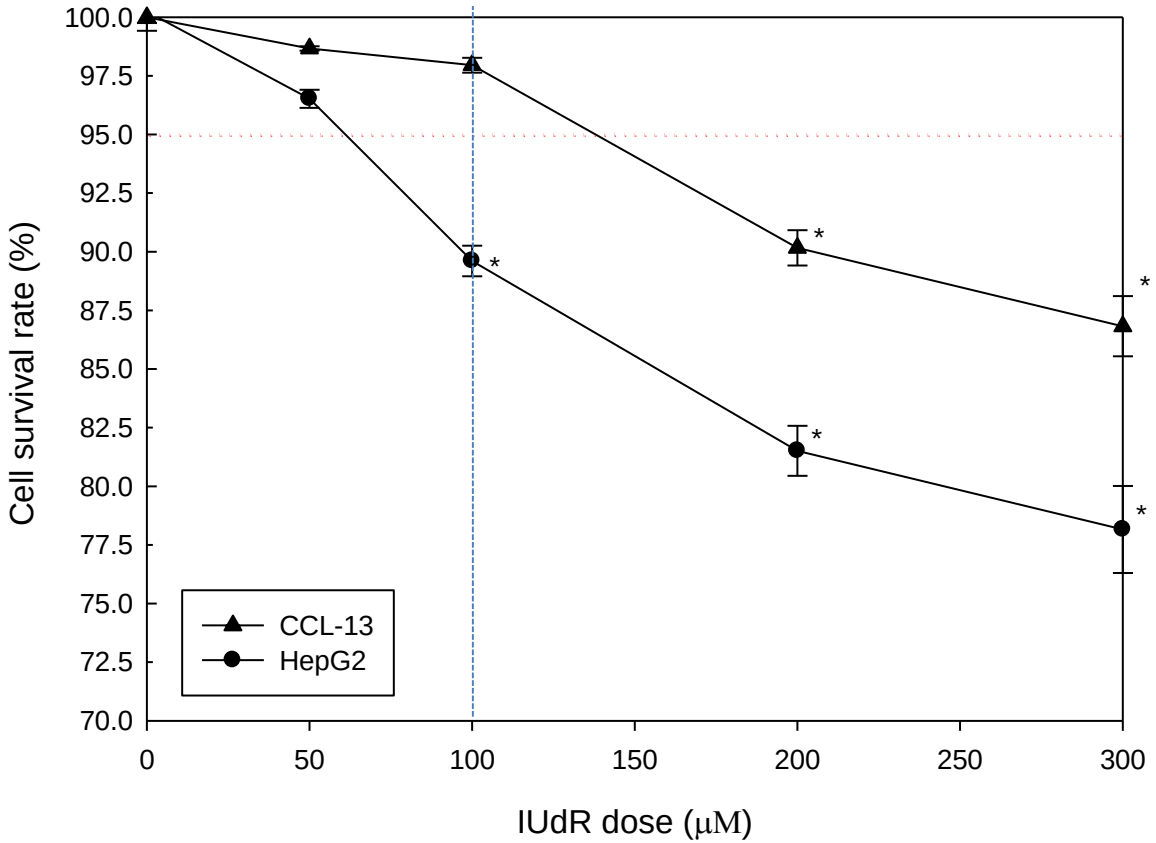
■ **5-iodo-2'-deoxyuridine (IUdR)**

➢ The van der Waals radius of an atom of iodine is very similar to that of a methyl group CH_3 (Prusoff, et al. 1979)

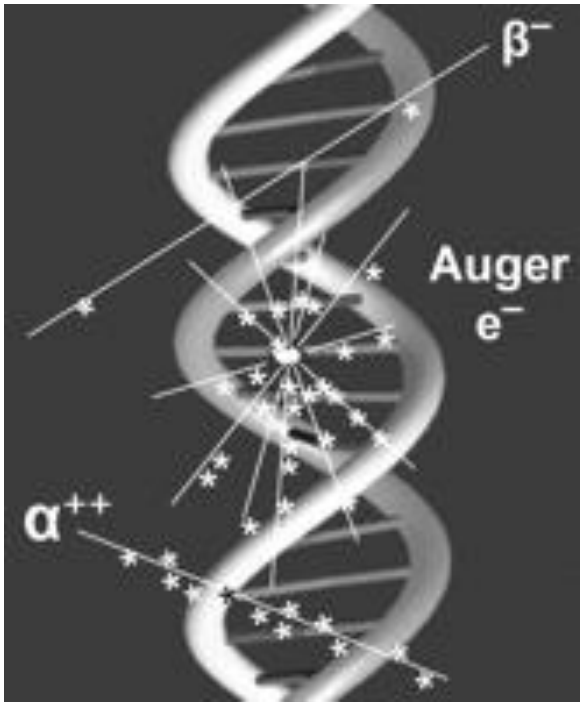


Thymidine (TdR) IUdR

- 使用放射性IUdR 於癌症治療另有一明顯優點，由於正常的細胞增殖緩慢，與迅速分裂增殖中的癌細胞比較，正常細胞對IUdR 的攝取率極低，可大幅提高放射性IUdR 作體內放射治療的tumor-to-normal ratio，這點對於整體器官輻射耐受性低如肝臟者尤為重要。



Auger Electron Emission



The auger electron emission following IUdR was highly toxic to mammalian cells and exceedingly efficacious in the therapy of small-animal malignancies.

IUdR 生理半衰期

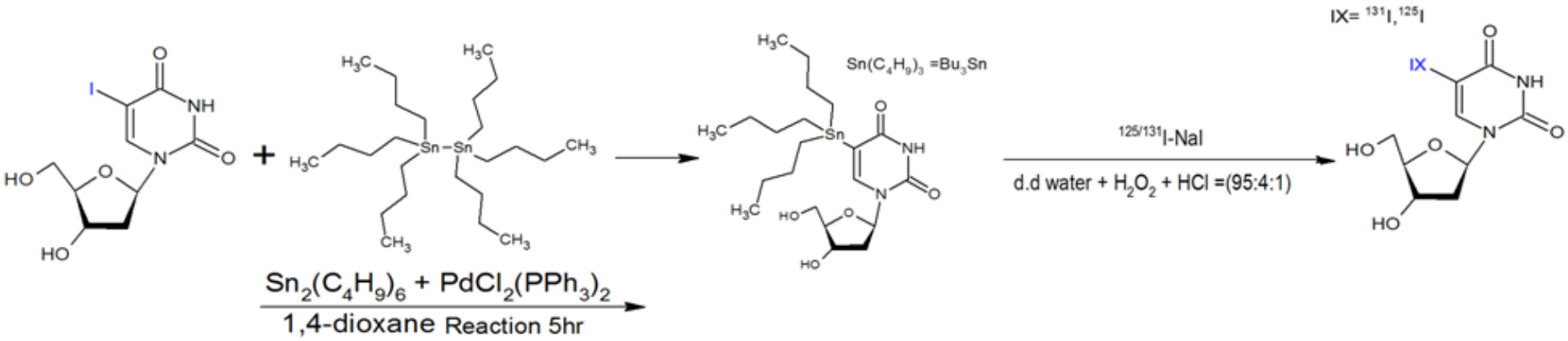
- IUdR 在活體內的生理半衰期甚短（在人體內僅為5分鐘，在老鼠則為七分鐘），目前研究結果大都指出必須將IUdR 直接注入腫瘤，或是由腫瘤上游動脈直接緩慢注射的給藥方式，方能被處於S期腫瘤細胞有效吸收；而未被腫瘤細胞吸收，殘留在體內的IUdR則迅速被代謝分解釋出碘離子由腎臟排出。

IUdR缺點

實驗目的

- 放射性藥物合成。
- 以微膠囊包覆藥物，延長藥物作用時間與增強歐傑電子劑量。
- 藥物生物分佈量測與動物實驗。

Part I - 放射性IUdR藥物合成



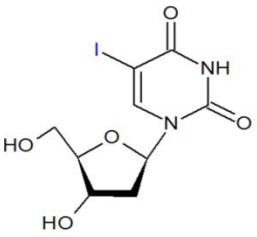
IUdR置換成Bu_3SnUdR前驅物反應

將IUdR溶解於1,4-Dioxane後，加入雙三丁基二錫$Sn_2(C_4H_9)$與二(三苯基膦)二氯化鈀$PdCl_2(PPh,C_6H_5)_2$，於溶液中加熱至95-98℃反應5hr，置換成Bu_3SnUdR，[$Bu_3SnUdR=(C_4H_9)_3SnUdR$]

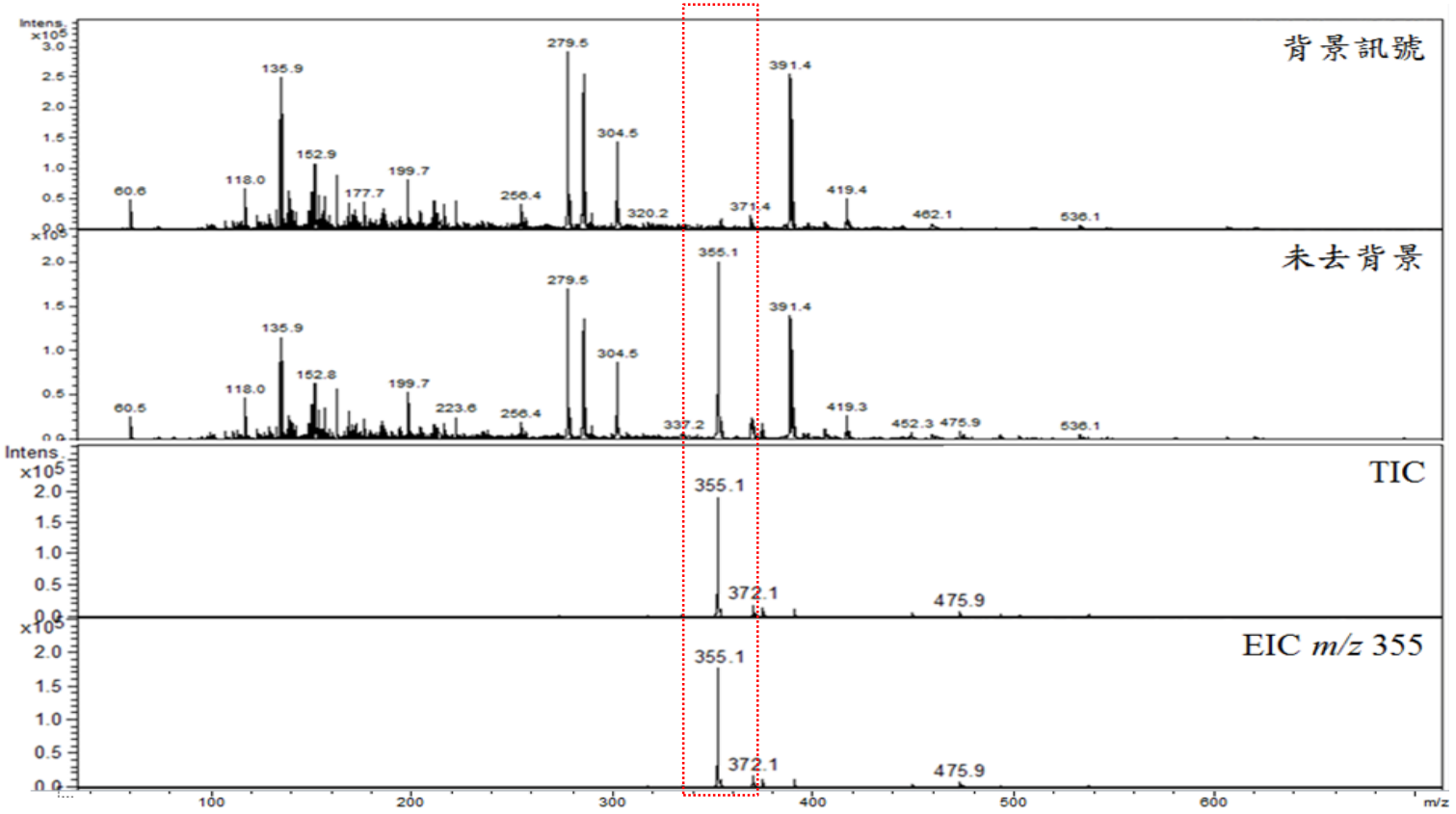
放射性IUdR反應合成

取Bu_3SnUdR前驅物加入放射性碘同位素與氧化劑進行碘化去錫反應，將放射性碘元素標誌成產物^{131}IUdR

ESI-MS analysis of IUDR

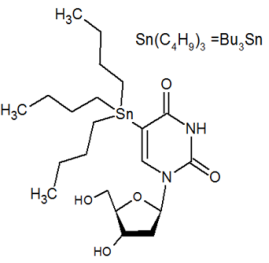


5-iodo-2-deoxyuridine
IUdR $C_9H_{11}IN_2O_5$
Molecular weight 354
355(m/z)

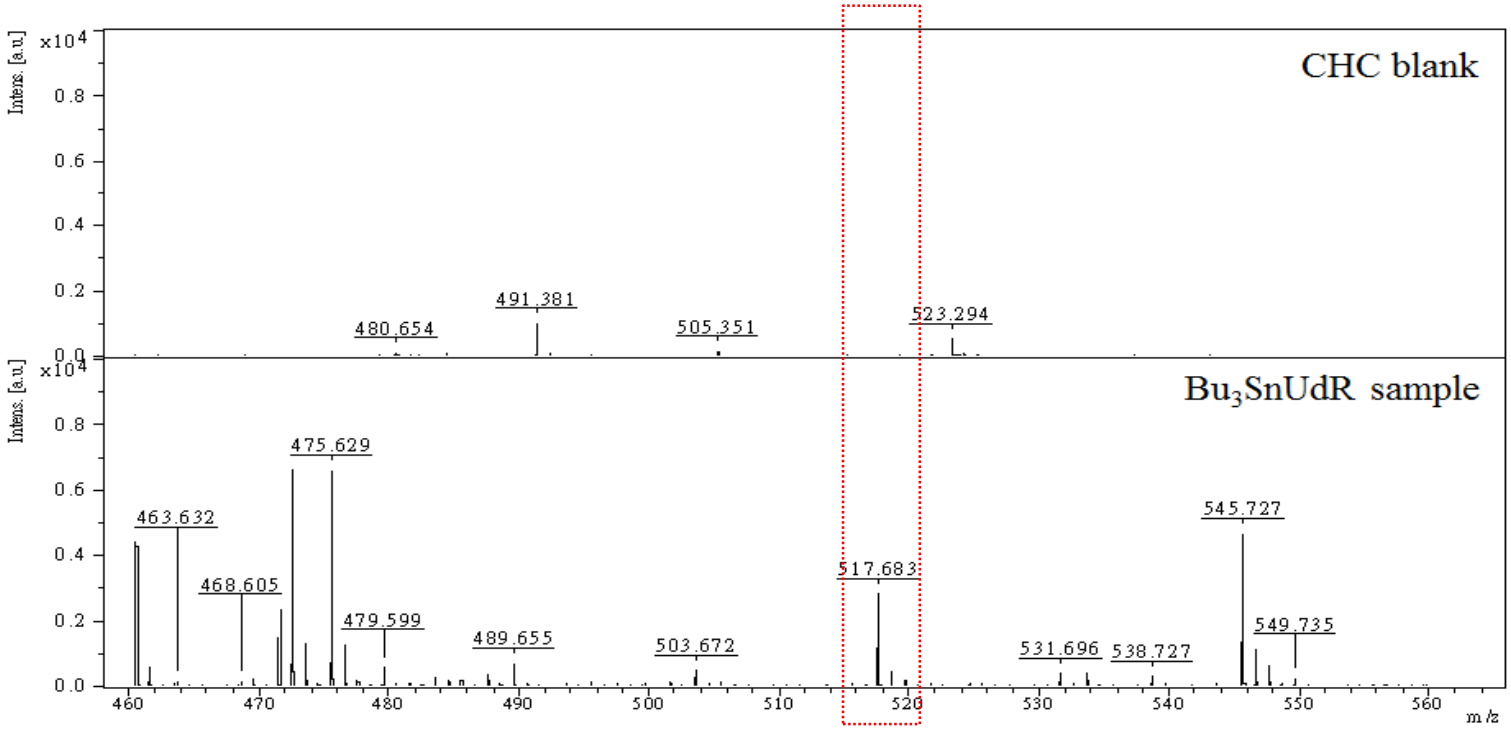


- Compare background and sample included background singnal,major peak appears at 355m/z
  TIC (Total Ion Chromatography), EIC (Extracted Ion Chromatograph)

MALDI-MS analysis Sn-UDR

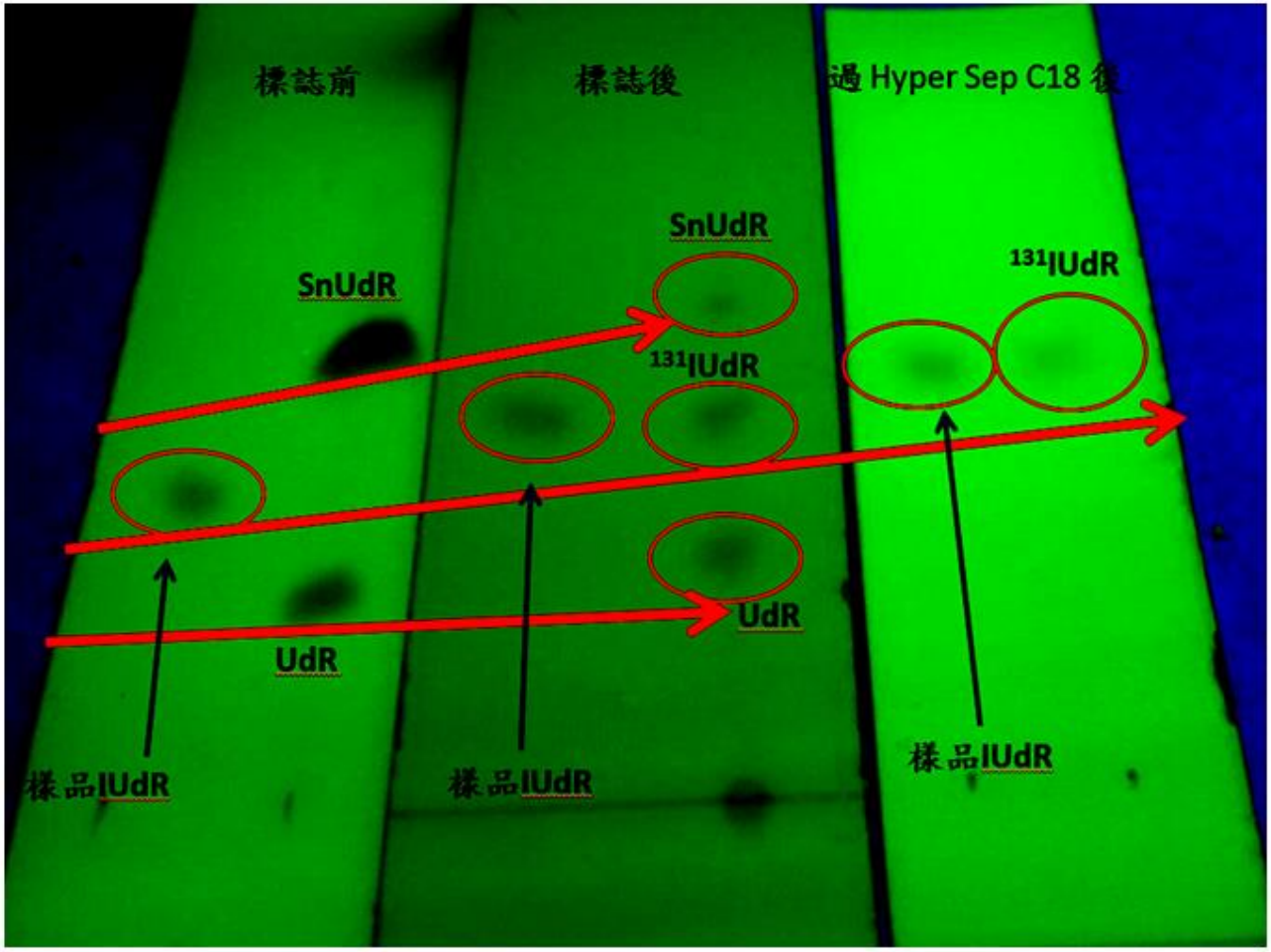


Bu_3SnUdR
$C_{21}H_{38}N_2O_5Sn$
Molecular weight 516.7



- Compared CHC (α-cyano-4-hydroxycinnamic acid) background signal and Bu3SnUdR sample signal, the main signal sample appears at 517 m/z with the signal range of 460-560m / z.

TLC分析放射性碘131-IUdR

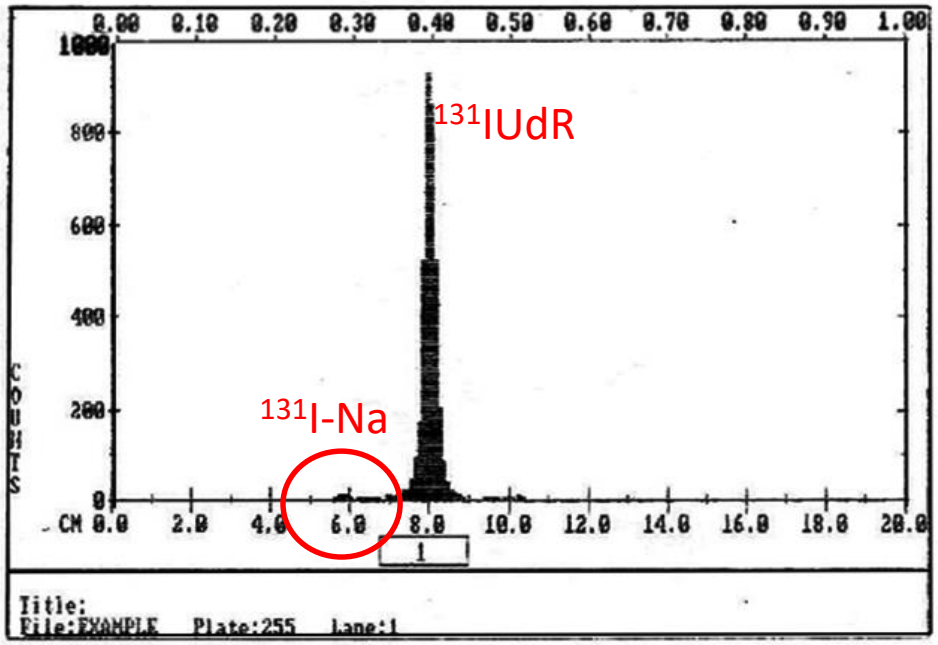


在每片TLC片的左側為對照的IUdR，(A)為未標誌前驅物SnUdR與游離的UdR，(B)為標誌合成後產物，由下往上分別表示為UdR, ^{131}IUdR,SnUdR)，(C)為過HyperSep C_{18}的純化^{131}IUdR

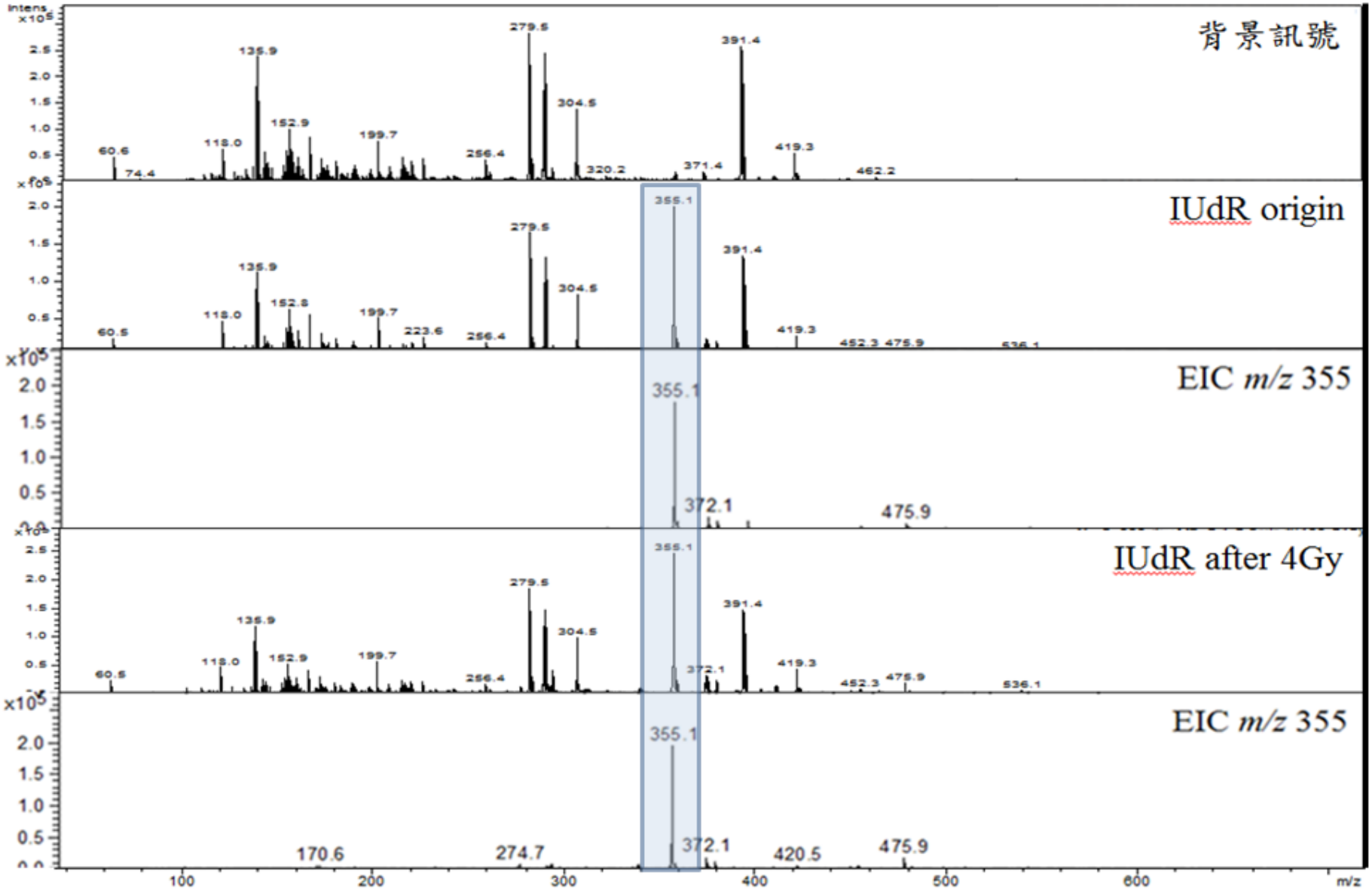
Radio TLC scanning

Total Count Region: 0.00cm to 20.00cm
Total Counts: 3939
Total CPM: 1970

Reg. #	Start (cm)	Stop (cm)	Center (cm)	Rf	Region Counts	Region CPM	% of Tot Reg	% of Tot Cnt
1	6.76	8.97	7.95	0.40	3659	1830	100.00	92.89
TOTAL					3659	1830	100.00	92.89

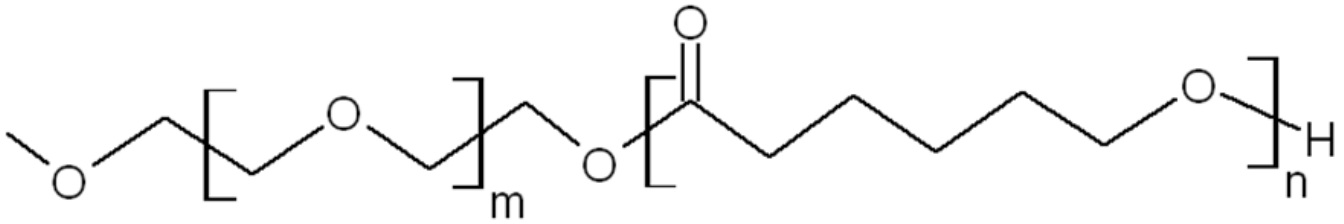


IUdR質譜分析



IUdR的訊號值為355m/z，比對IUdR照射4Gy後，無明顯分子改變的訊號。

Part II - 微膠囊包覆藥物



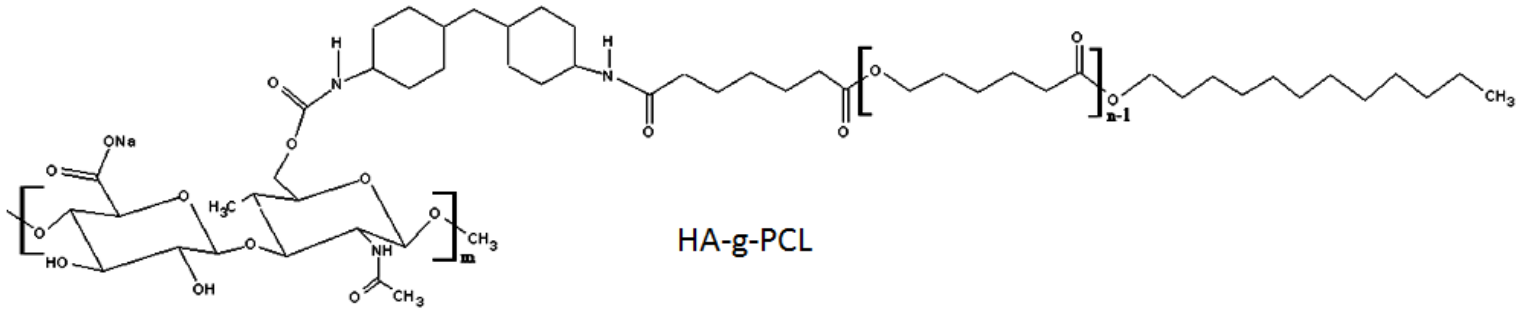
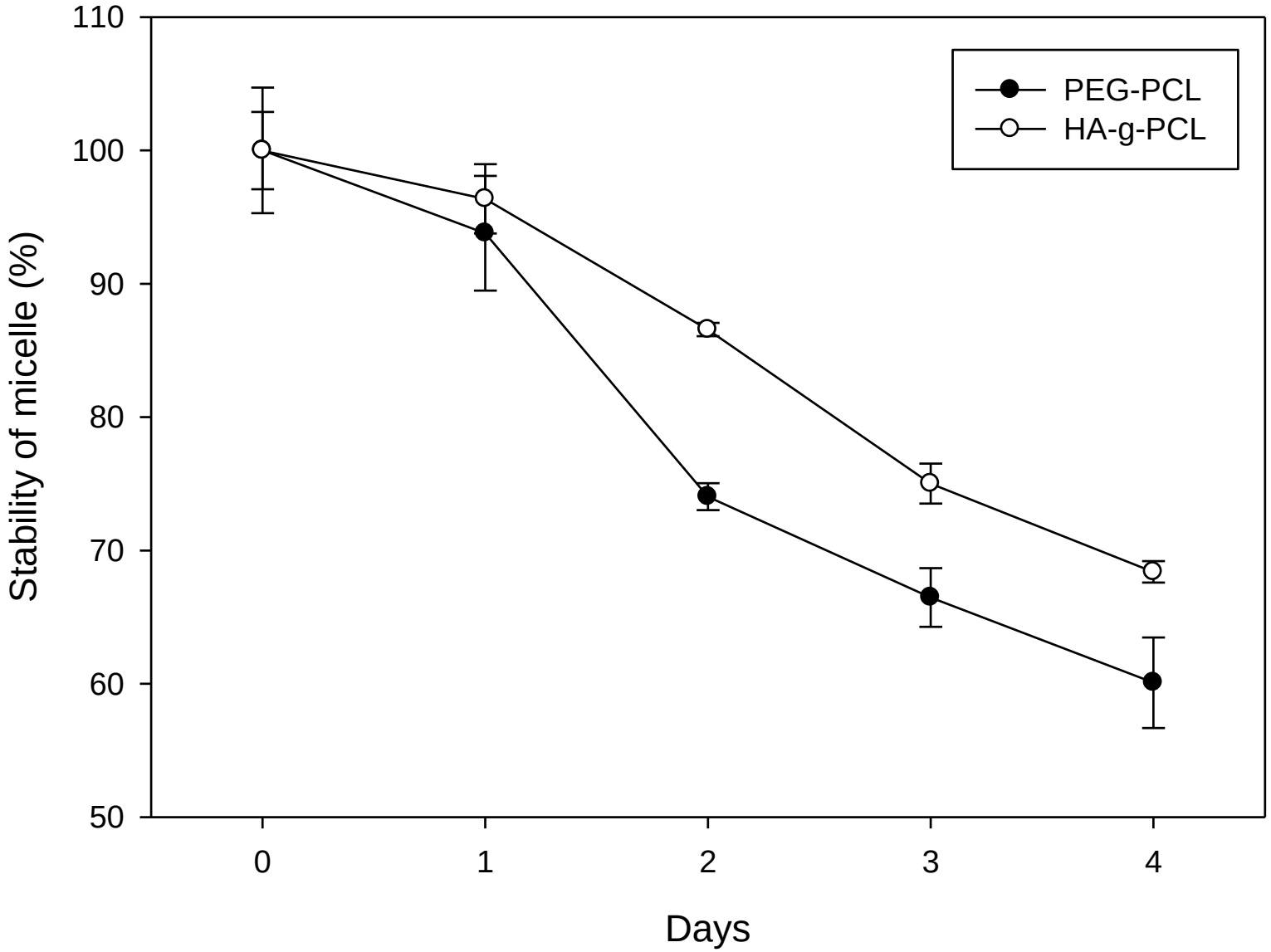
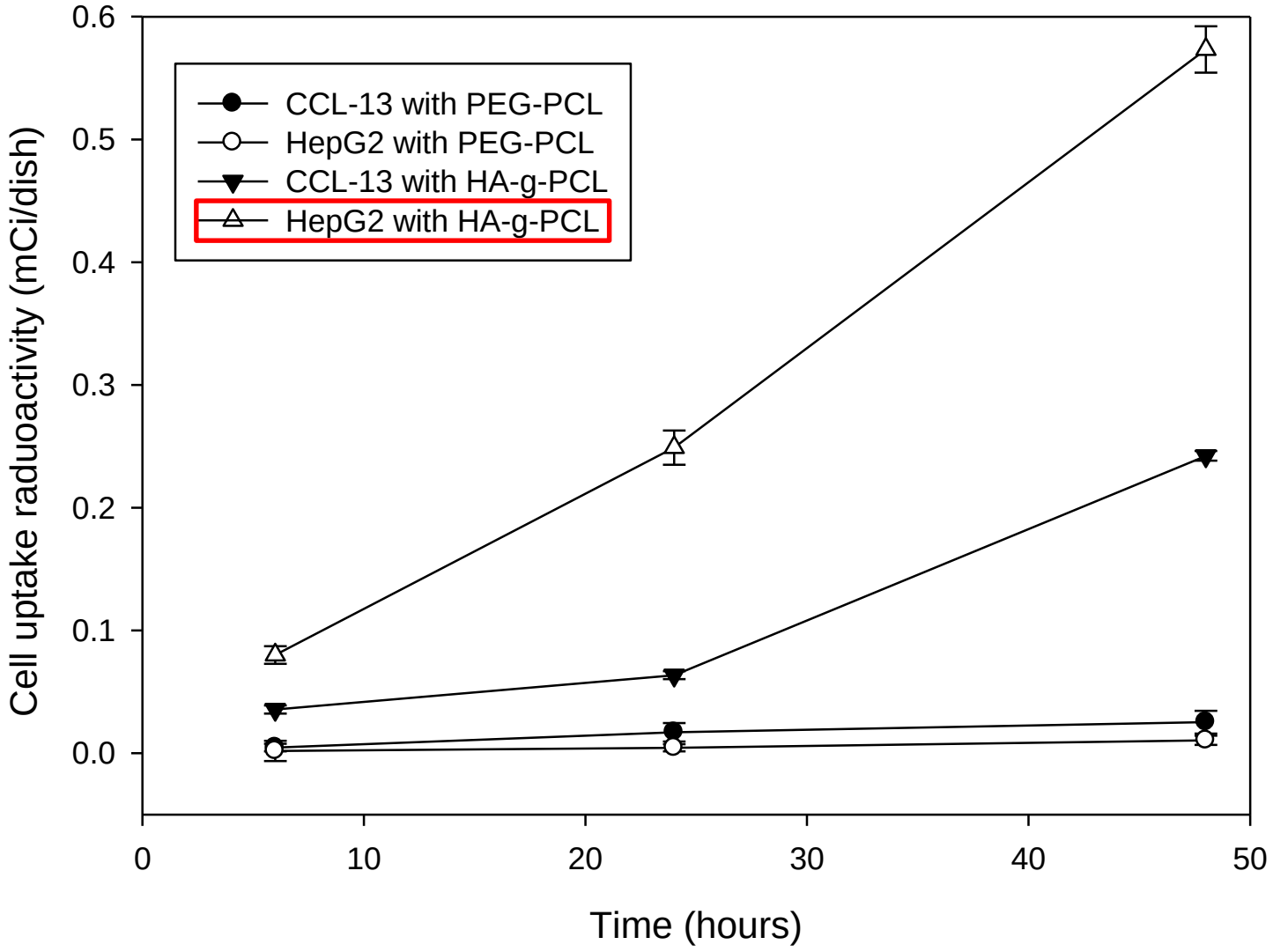


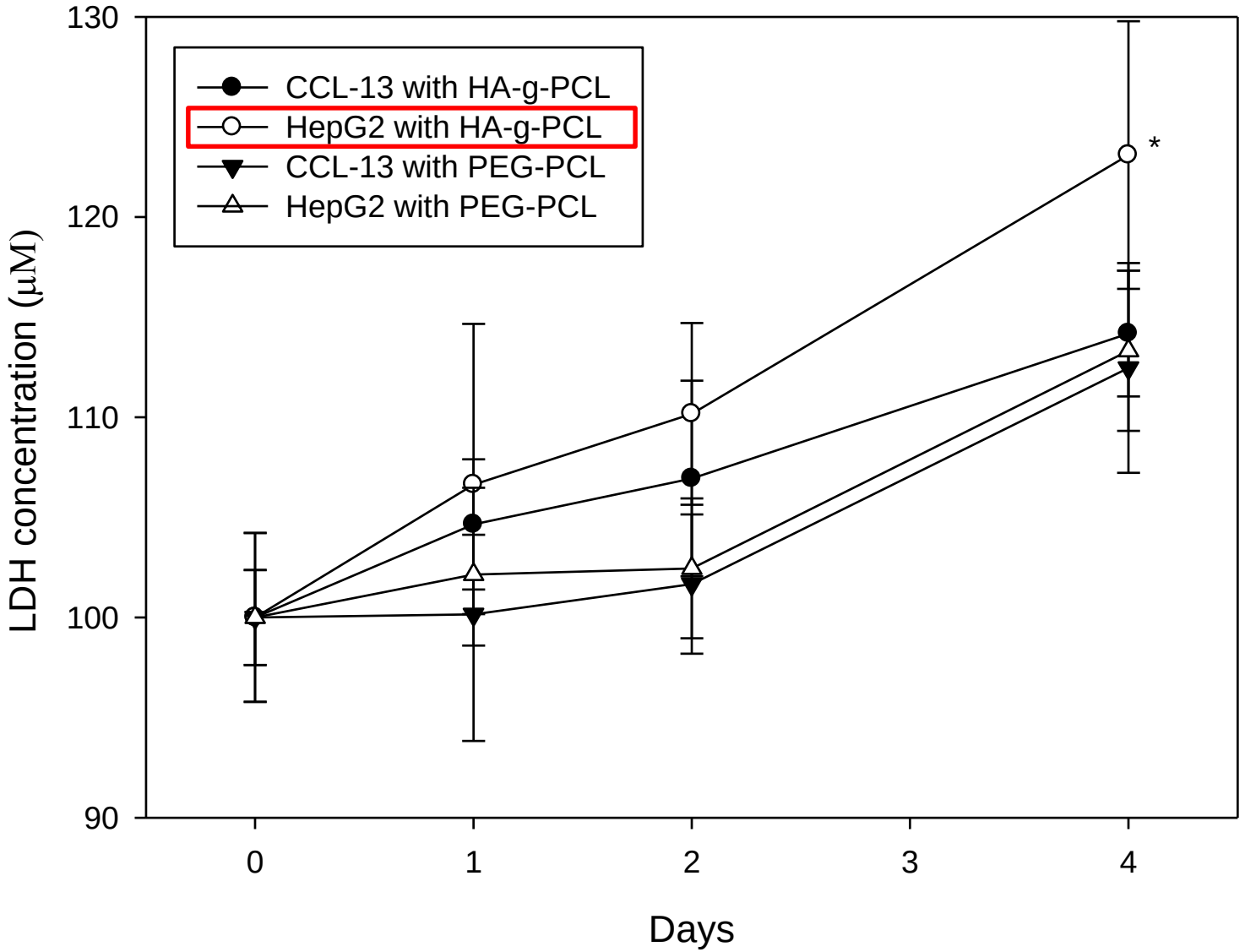
Figure 1. The chemical structures of PEG-PCL and HA-g-PCL monomers.



The stability of ^{131}IUDR loaded micelles. In this study, it was shown that both ^{131}IUDR loaded HA-g-PCL and PEG-PCL micelles kept their integrities well, holding as high as 60% ^{131}IUDR in the micelles after 4 days.

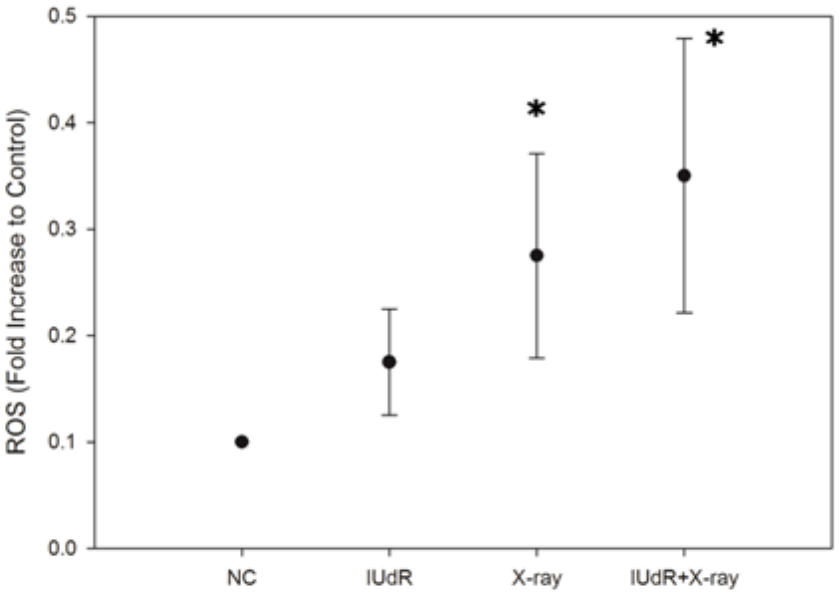


The cell uptake of the micells encapsulated with ^{131}IUDR. The HepG2 showed better uptake of ^{131}IURD delivered by HA-g-PCL micelles than CCL-13 cells did.

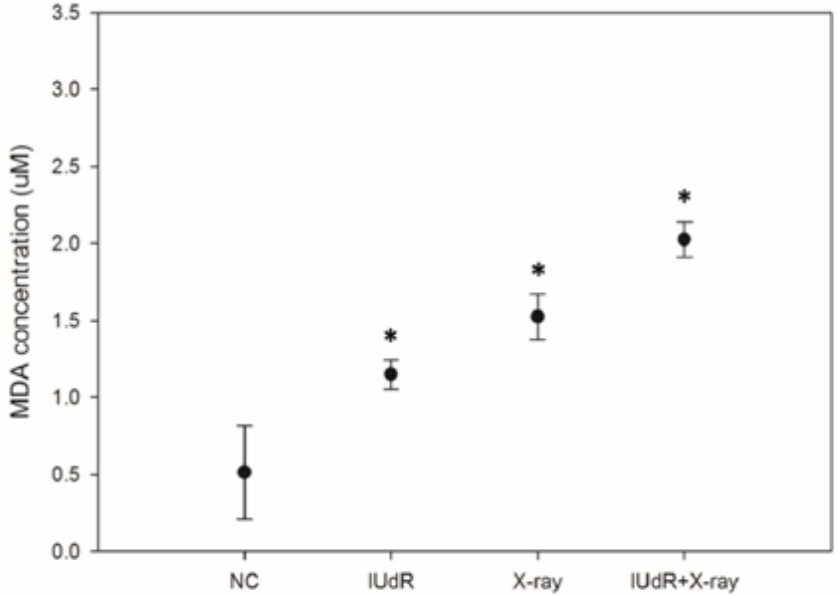


The cytotoxicity of the micells encapsulated with ^{131}IUDR. After 4 days incubation, the LDH concentrations of HepG2 cells were increased significantly. It may be due to the increased cell uptake activity and higher stability of ^{131}IUDR loaded HA-g-PCL micelles in which contributed to have better ^{131}IUDR control release rates.

Result of ROS and MDA



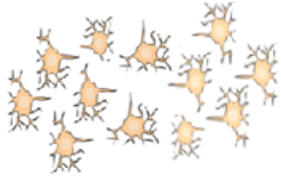
(n=32) (✻ : P < 0.05, t-test)



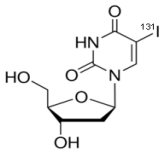
(n=32) (✻ : P < 0.05, t-test)

The expression of ROS and MDA will be increased with IUdR and X-ray.

Part III – 動物實驗與生物分佈

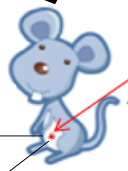


4T1 cell injection

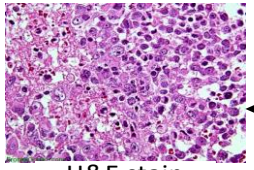


IUdR injection

Tumor formation

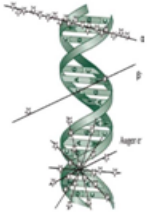


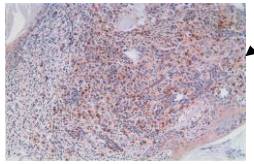
Nano-ray



H&E stain

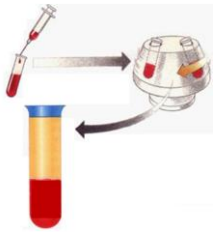
Auger emission



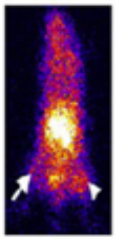


immunohistochemistry
(IHC stain)

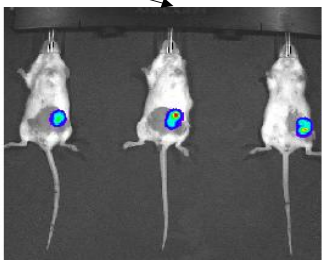
Survival



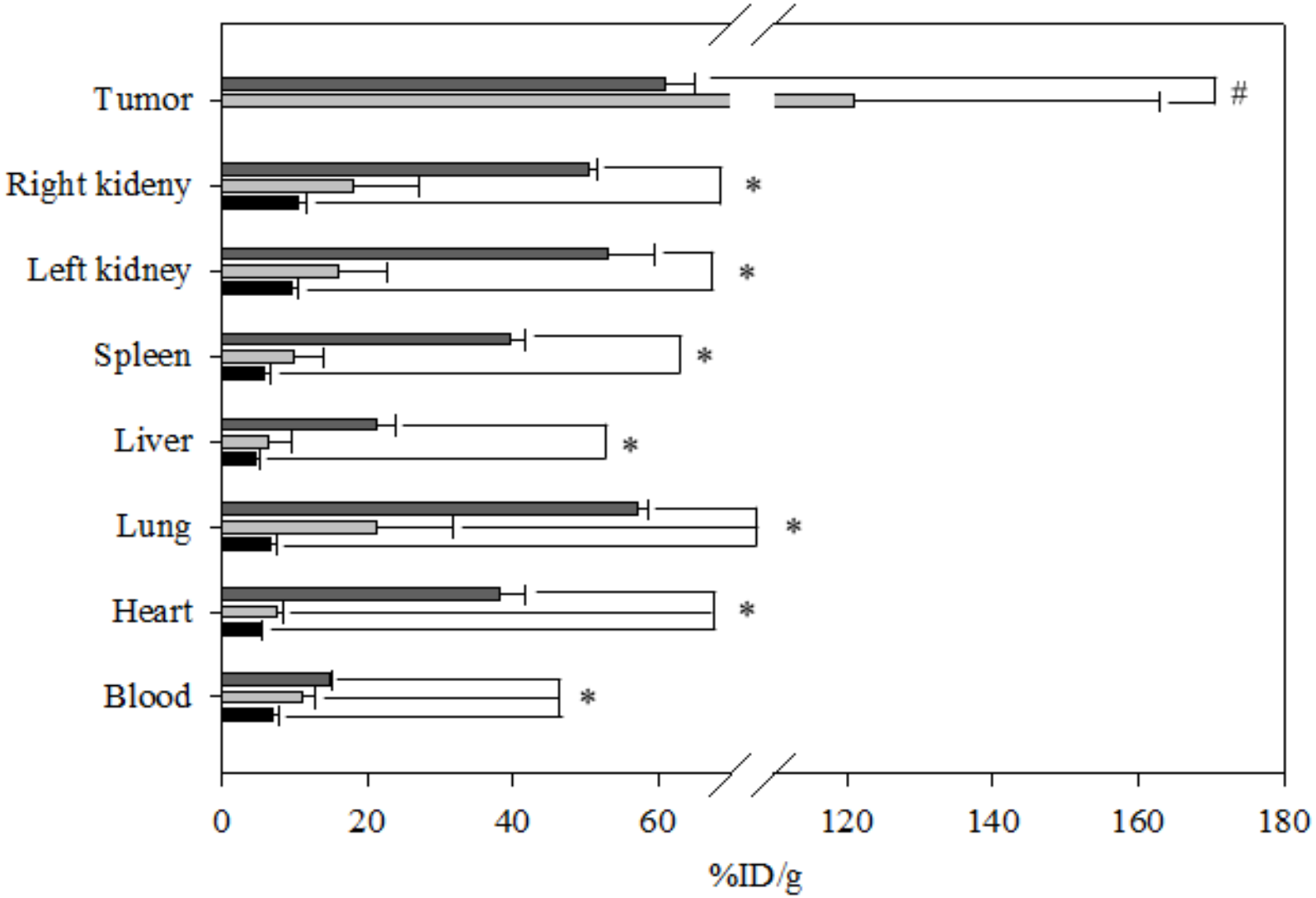
Blood / Serum
test

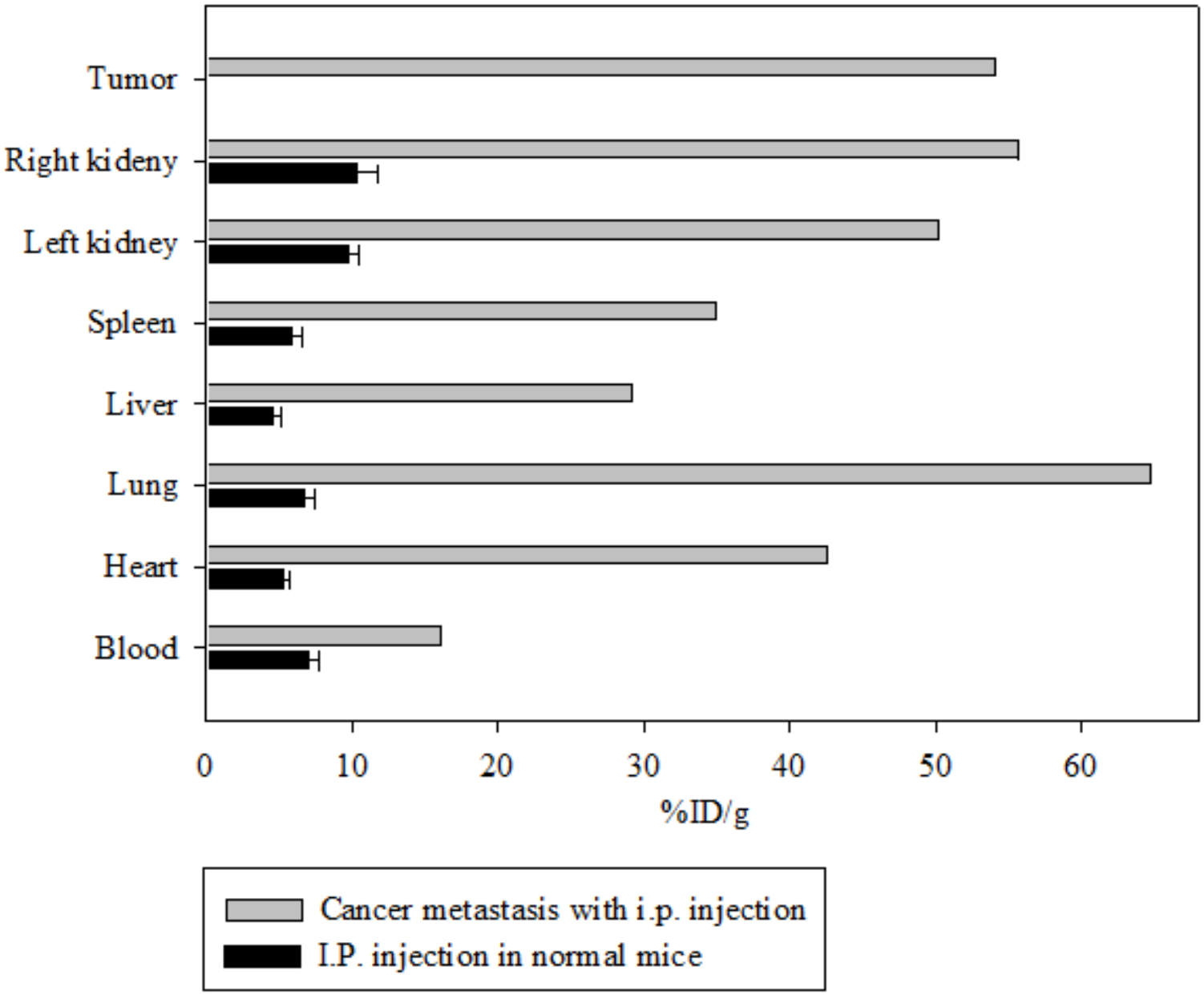


Biodistribution of
IUdR in mice



IVIS image of tumor


Tumor
Right kideny
Left kidney
Spleen
Liver
Lung
Heart
Blood
#
*
0
20
40
60
120
140
160
180
%ID/g
Intraperitoneal injection
Intratumoral injection
Intraperitoneal injection in normal mice


Tumor
Right kideny
Left kidney
Spleen
Liver
Lung
Heart
Blood
0
10
20
30
40
50
60
%ID/g
Cancer metastasis with i.p. injection
I.P. injection in normal mice

^{131}I-IUdR小鼠器官生物分佈

Tissue	Mean(%)±SE	%ID/g
Blood	4.52±0.004	6.96±0.006
Thyroid	0.75±0.004	32.27±0.046
Heart	0.77±0.000	5.28±0.003
Lung	1.15±0.002	6.8±0.005
Liver	6.1±0.008	4.63±0.007
Spleen	0.91±0.001	5.86±0.01
Left kideny	1.73±0.002	9.73±0.007
Right kideny	1.89±0.001	10.39±0.008
Tumor	12.71±0.069	120.99±0.423

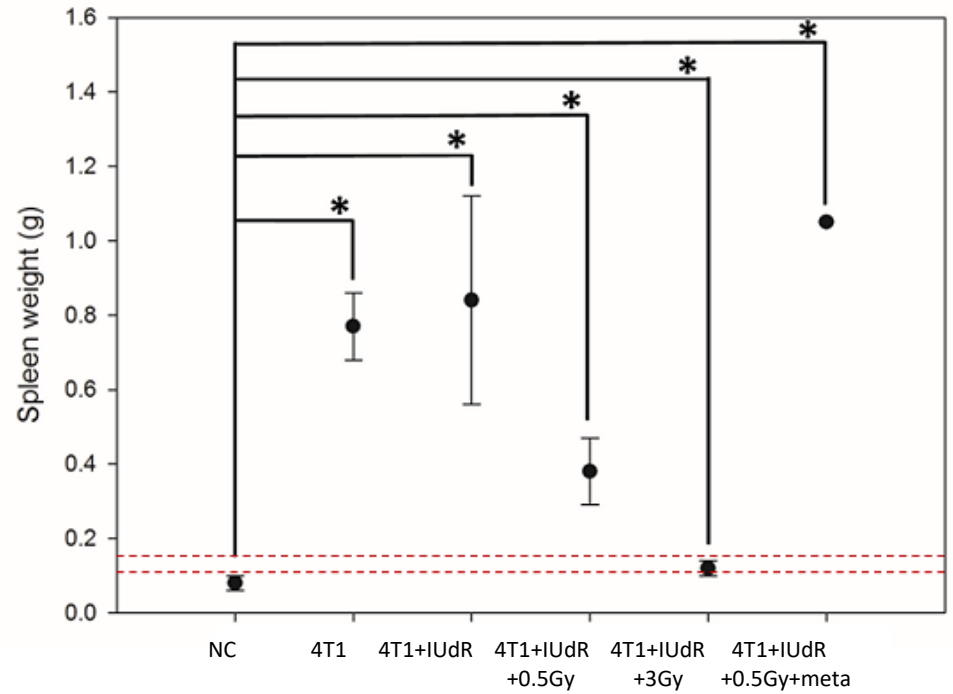
組織器官之放射性活度生物分布以每克組織重之百分注射放射藥物活度為單位(%ID/g)表示，對於重量小於1 g的組織器官具有極高劑量聚積時，%ID/g即可能會大於100%。

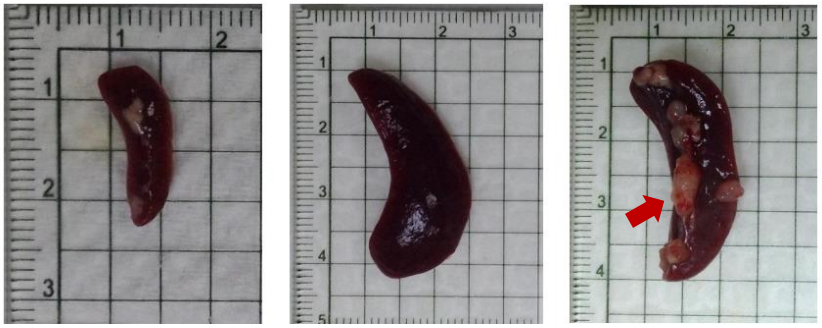
$$\%ID/g = C_T \times \frac{V_T}{W_T} \times \frac{1}{D_{inj}} \times 100\%$$

The results of serum tests in mice animal model.

	AST	ALT	CREA	Spleen weight
Normal	255.23±61.39	71.46±14.61	0.15±0.03	0.08±0.01
4T1	256.08±39.96	62.75±15.82	0.16±0.02	0.77±0.08*
4T1+IUdR	260.33±12.87	56.57±6.76	0.15±0.02	0.84±0.16*
4T1+IUdR+ 2 Gy	387.28±87.40* ↑	76.94±17.11	0.13±0.02	0.38±0.07* ↓

The weight and size of spleen





(Normal) (4T1+IUdR) (meta)

(n=22) (＊：P<0.05, t-test)

Normal mice : 0.130 ± 0.02 g

Anemia is a common complication of cancer; a role of spleen in tumor-stress erythropoiesis has been suggested.

The tumor development blocks medullar erythropoiesis by granulocyte colony-stimulating factor and then causes anemia in murine 4T1 breast tumor-bearing mice.

2 Gy X-ray輻射照射對小鼠造血功能之急性影響

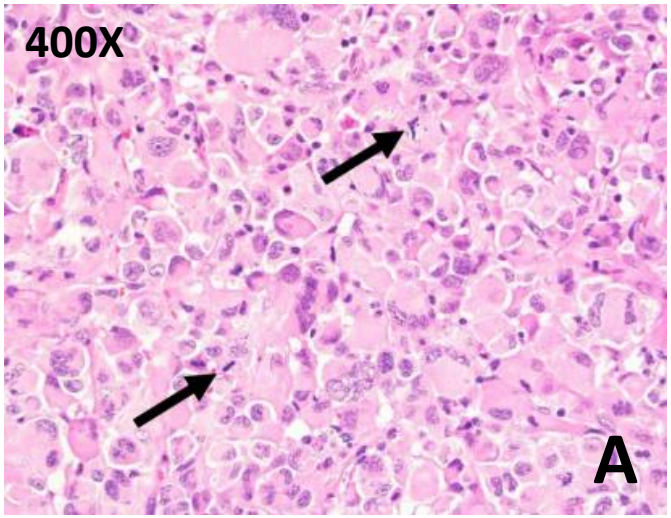
小鼠全套血球計數（Complete Blood Count；CBC）

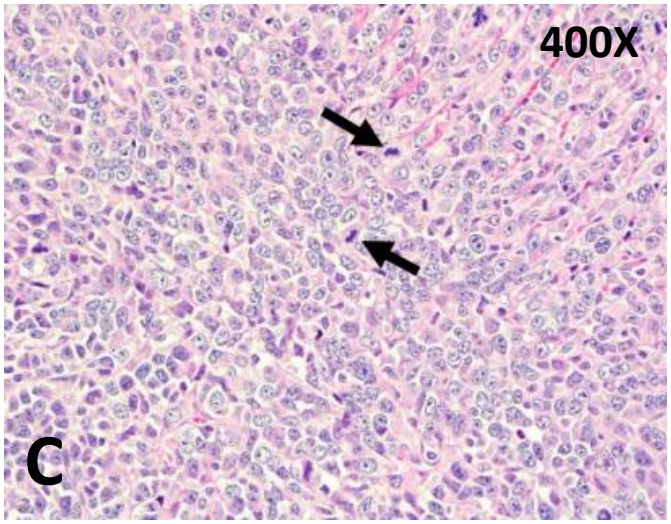
	Units	實驗組	正常值
RBC	$x10^6$ cells/μL	6.92 ± 0.42	10.5 ± 0.25
%RET	%	3.73 ± 1.17	2.98 ± 0.45
abs_ret	$x10^3$ cells/μL	348.65 ± 40.04	307 ± 42

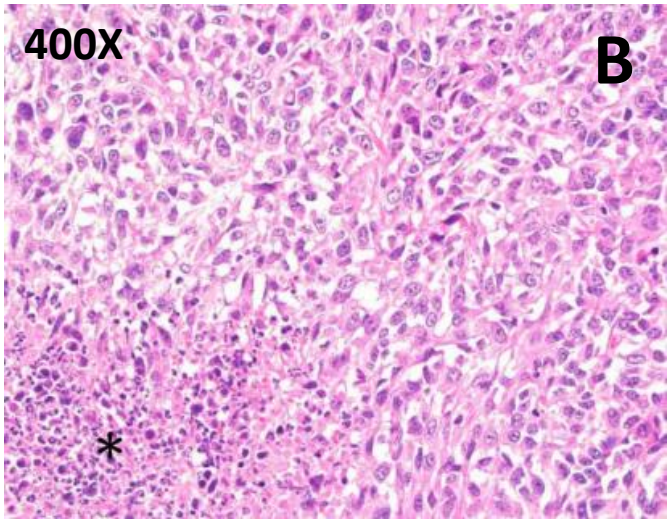
縮寫與全名寫：RBC(red blood cells), %(percent), abs (absolute counts), RET (reticulocyte)

使用IDEXX ProCyte Dx 血液學分析儀及相關試劑進行分析

Histologic analysis of tumor







(A) Tumor cells reveal cellular pleomorphism, prominent nucleoli with abundant eosinophilic cytoplasm and few mitoses (arrows). 細胞多形性，有絲分裂（箭頭），核仁明顯。

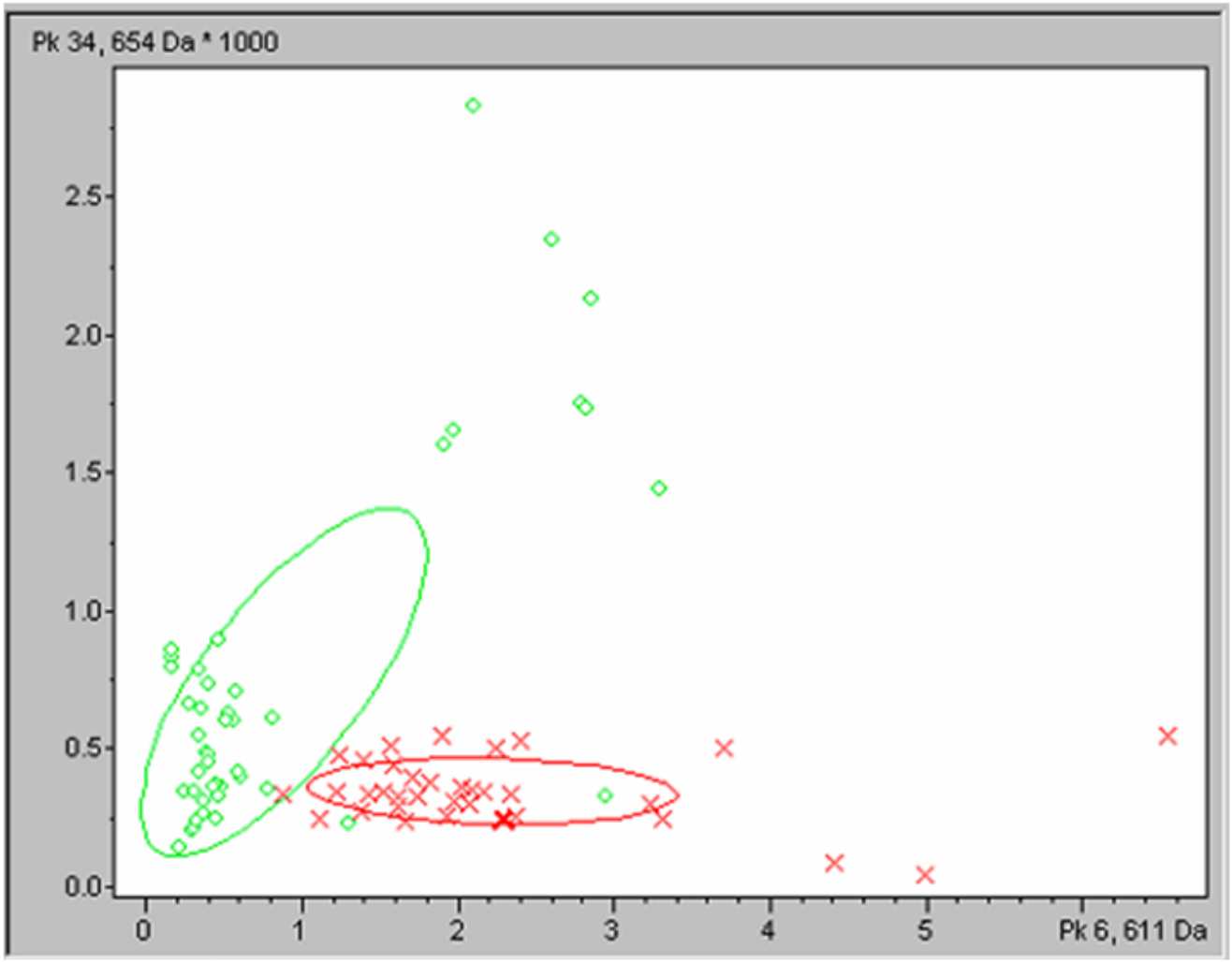
(B) 4T1+IUdR+0.5Gy : The tumor grows in solid sheet with variable size and focal necrosis (*).局部性壞死。

(C) 4T1+IUdR+0.5Gy+meta : The tumor grows in solid sheet with variable size and few mitoses (arrows).腫瘤生長並有絲分裂。

Proteomic study – 4T1 cell Control v.s. IUdR+1 Gy

- MALDI MS analysis: 149 signal with significal difference.
- Compared to LC-MS/MS protein ID, which were fit to 40 amino acid sequences.
- Experimental results reported a total of 36 protein identifications with higher confidence levels.
- Protein-protein interaction analysis – 11 proteins with connection.

Proteomic - PCA analysis

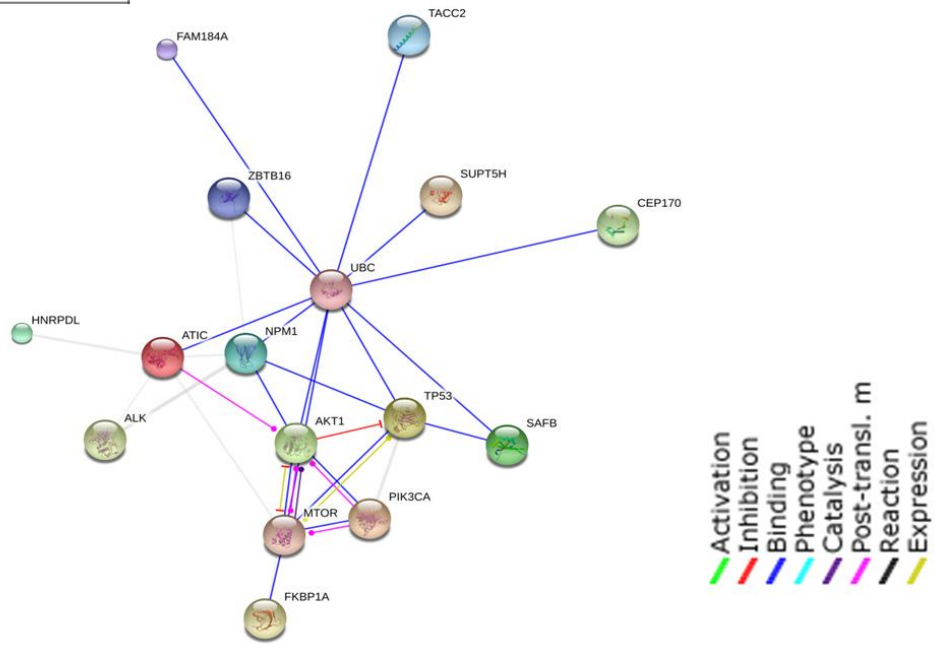


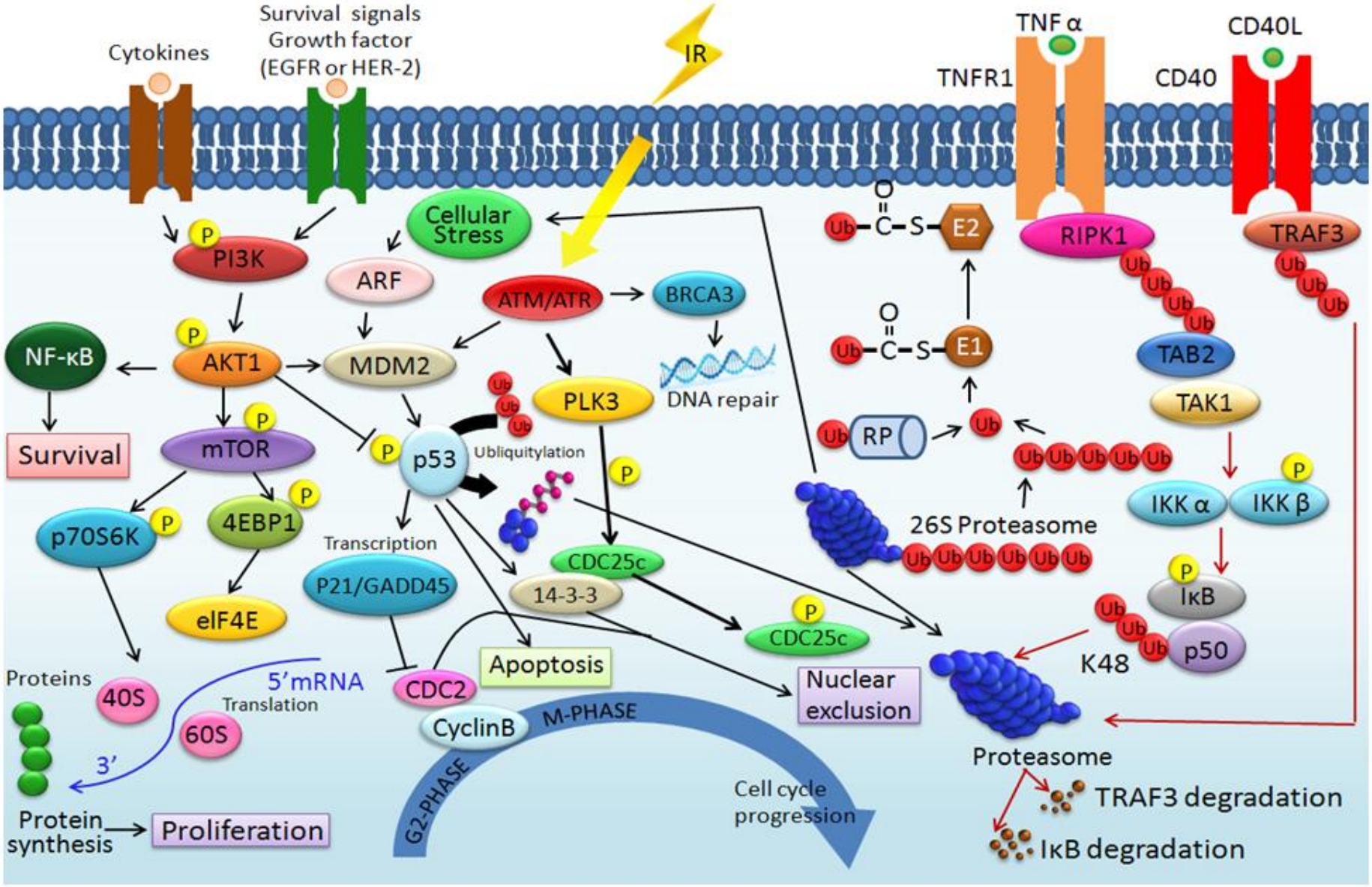
Green – +IUDR+X-ray, Red - control

String 9.1 protein-protein interaction analysis

ALK	**Q9UM73**	Adenylate kinase domain-containing protein 1
ATIC	**P31939**	ALK tyrosine kinase receptor
CEP170	**Q5SW79**	Centrosomal protein of 170 kDa
HNRPDL	**O14979**	Heterogeneous nuclear ribonucleoprotein D-like
NPM1	**P06748**	Nucleophosmin
FKBP1A	**P62942**	Peptidyl-prolyl cis-trans isomerase FKBP1A
FAM184A	**Q8NB25**	Protein FAM184A
SAFB	**Q15424**	Scaffold attachment factor B1
SUPT5H	**O00267**	Transcription elongation factor SPT5
TACC2	**O95359**	Transforming acidic coiled-coil-containing protein 2
ZBTB16	**Q05516**	Zinc finger and BTB domain-containing protein 16

Protein map by String 9.1





Schematic representation of some possible signaling pathways activated by IUdR with X-ray irradiation which may regulate metabolism of proliferating cells.

現有動物肝癌模式
GNMT-/-基因剔除鼠
HBx基因轉殖鼠

研究室成員

黃琬玲(屏科大動物碩士)

陳宛柔(清大原科博士)

致謝 高醫-中山合作計畫〈4年80萬〉

NO	計畫名稱	計畫編號	起迄日期
1	放射性碘去氧尿嘧啶於乳癌治療-細胞吞噬與小鼠生物分布之研究	NSYSUKMU105-P032	105/01/01 105/12/31
2	Iododeoxyuridine (IUdR)輻射增敏應用於肝癌治療之動物實驗	NSYSUKMU104-P032	104/01/01 104/12/31
3	癌症標靶治療微胞藥物開發-輻射增敏劑應用於歐傑電子治療之研究	NSYSUKMU103-P006	103/01/01 103/12/31
4	CD44抗體作為包覆放射性碘131-Lipiodol微胞囊表面修飾之標靶探針並評估對肝癌細胞之專一性.	NSYSUKMU102-P006	102/01/01 102/12/31

Thanks for your attention