出國報告（出國類別：開會）

2024年歐洲泌尿科年會報告、討論過程及考察心得

服務機關：成大醫院泌尿部
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派赴國家/地區：法國巴黎
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報告日期：2024/4/6

摘要

目的：探討來自癌細胞的外泌體中 Prothymosin-α (ProT)在促進肌肉消耗中的潛在相關性。

方法：我們分析膀胱癌患者的血清樣本，比較了患有惡病質和無惡病質的患者之間的 ProT 和 IL-6水平。隨後，從小鼠膀胱癌 MBT-2細胞中分離出外泌體，並對這些外泌體內與細胞外液 ProT 含量做比較。然後，將肌肉細胞暴露於來自 MBT-2的外泌體中，並測量 TGF-β 、IL-1β和 IL-6的表現。我們另外使用 ProT 基因敲除的 MBT-2細胞的外泌體進行實驗，並研究外泌體中通過 TLR-4活化 NF-κB 的分子機制。

結果：在患有惡病質的膀胱癌患者血清中 ProT 和 IL-6水平較無惡病質高。在 MBT-2細胞中，ProT 在外泌體中富集，而非細胞外液。MBT-2細胞的外泌體會使肌肉中與消耗相關的細胞激素增加。ProT 基因敲除會逆轉此現象。外泌體中的 ProT 會通過 TLR-4在肌肉細胞中活化 NF-κB，導致細胞激素表現增加。在小鼠模型中，癌細胞 ProT 基因的敲除改善了小鼠的體重下降和肌肉萎縮。

結論：癌細胞外泌體中的 ProT 與肌肉細胞的相互作用可能為惡病質提供了治療前景。

目次

本文

目的：將本實驗室目前對膀胱癌惡病質的研究情形，於2024年歐洲泌尿科年會中和與會醫師及學者進行討論及分享。

過程：

簡介：本次歐洲泌尿科年會於2024年4月5日至8日(全日)於法國巴黎”Paris Expo Porte de Versailles”會場舉行，與會者為來自全球各地的泌尿科主治醫師、住院醫師、相關醫療人員如護理師等等，以及眾多介紹自家最新醫療科技及藥物的廠商，可說是每年一次的世界級盛會。本次報告的內容歸屬在”Basic research and trials: Oncology”，為Expert-Guided-Poster-Tours (EGPT)，需有至少五分鐘的演講及兩分鐘的討論時間。

本場次的主席及相關的討論題目：

Saturday 6 April **15:15 - 16:45**	**Location** Purple Area, EGPT **Chairs** R.T. Bryan, Birmingham (GB) G. Niegisch, Düsseldorf (DE) We encourage participants in this session to view the abstracts before the session. All abstract bodies, posters, videos and webcasts can be viewed on the EAU(N)24 Resource Centre or at the digital screens in front of the Purple Area, EGPT.
15:15 - 15:27	**Screen A: Bladder cancer**
P146	**Urinary proteomics for microbiome identification and its implication in bladder cancer pathogenesis** P.V. Stamatakos, Piraeus (GR)
P164	**Bladder cancer organoids serve as a useful system to mirror the characteristics of parental tumours** H. Zhao, Hongkong (HK)
P172	**A zebrafish xenograft model for evaluating efficacy of cisplatin in muscle-invasive bladder cancer** Y. Sugino, Tsu (JP)
P155	**Low-absent expression of TROP-2 in sarcomatoid subtype of bladder cancer: immunohistochemical evaluation of TROP-2 in a series of muscle invasive bladder cancer** G. Giannarini, Udine (IT)
15:27 - 15:39	**Screen B: Bladder cancer**
P163	**Expression of CYTOR in Cancer-Associated Fibroblasts Is Associated with the Prognosis and Immunotherapeutic Response in Bladder Cancer** Y. Wu, Beijng (CN)
P152	**Combined analysis of molecular patterns and clinical factors for the prediction of immune checkpoint inhibitor therapy in advanced urothelial carcinoma** T. Szarvas, Budapest (HU)
P171	**Cancer-derived exosomal prothymosin-α triggers muscle wasting via regulating TLR4-NF-κB-E3 ubiquitin ligase signaling pathway** C-Y. Hu, Tainan (TW)
15:39 - 15:54	**Screen C: Bladder cancer**
P149	**PHF6 promotes bladder cancer malignant progression via facilitating P53 ubiquitination** Q. Zhang, Nanjing (CN)
P154	**AIRD1A Knockouts in Bladder Cancer Cell Lines Leads to MYC and E2F Activation Implicated in More Aggressive Tumor Behavior** C.J. Magnani, Boston (US)

P153	**The m6A reader IGF2BP3 promotes bladder cancer progression via enhancing HSP90AB1 expression** Q. Zhang, Nanjing (CN)
P159	**The histone methyltransferase KMT9 regulates proliferation and migration of bladder cancer cells and is a novel target for treatment of muscle invasive bladder cancer (MIBC)** S. Totonji, Freiburg (DE)
P158	**YTHDC1 reduces M2 macrophage infiltration by regulating tumor senescence to suppress lymph node metastasis of bladder cancer** Y. Su, Qingdao (CN)
15:54 - 16:09	**Screen D: Prostate cancer**
P160	**Generation of a new transgenic mouse model to explore the role of oncofetal CRIPTO in lethal prostate cancer** G. Thalmann, Bern (CH)
P150	**Investigating prostate cancer cellular heterogeneity and treatment response at single-cell level** R. Parmentier, Basel (CH)
P169	**Exploring the genomic blueprint of de novo neuroendocrine prostate cancer: Insights for innovative therapies from spatial gene expression analysis** R. Watanabe, Seattle (US)
P162	**Melatonin Induces P53 Phosphorylation to Enhance SLC7A11-Mediated Ferroptosis and Inhibit Prostate Cancer Progression** H. Huang, Guangzhou (CN)
P161	**Androgen receptor mediated tumor-associated macrophages drives prostate cancer progression to castration-resistant prostate cancer.** H. Huang, Guangzhou (CN)
16:09 - 16:21	**Screen E: Prostate cancer**
P151	**Enzalutamide (enza) with or without leuprolide in patients (pts) with European Association of Urology (EAU)-guideline-defined high-risk biochemically recurrent prostate cancer (BCR) following radical prostatectomy (RP) or radiation therapy (RT): EMBARK post hoc analysis** U. De Giorgi, Meldola (IT)
P168	**Uncovering somatic genetic susceptibility factors in prostate cancer through comprehensive genome-wide analysis** L. Lin, Chengdu (CN)
P148	**Unveiling the Hidden Role of Spermine: Targeting HMOX1-induced Mitophagy in Prostate Cancer Therapy** J. Sun, Hong Kong (HK)
P166	**T cells subset in tumor-draining lymph nodes and blood from patients with prostate cancer** A.Z.S. Saudi, Norrköping (SE)
16:21 - 16:36	**Screen F: Kidney cancer**
P167	**Targeting glutamine addiction to induce ferroptosis with potent drug combination therapy for renal cell carcinoma** A. Takeuchi, Aichi (JP)
P156	**Carbonic anhydrase IX in renal cell carcinoma: a relevant biomarker and promising therapeutic target** J. Breza Sr., Bratislava (SK)
P157	**Prognostic role of PD-L1 in tumor tissue and liquid biopsy in clear cell renal cell carcinoma** P. Zeuschner, Homburg (DE)
P170	**Effect of HLA genotype on anti-PD-1 antibody treatment for advanced renal cell carcinoma** T. Tanegashima, Fukuokashi Higashiku (JP)
P147	**Metallothionein 1 X is a tumor suppressor gene and inhibits metastasis in clear cell renal cell carcinoma** R. Rui, Beijing (CN)

報告內容及相關討論：

Our study aims to investigate the potential role of cancer-derived exosomal ProT in promoting cachexia-related muscle wasting.

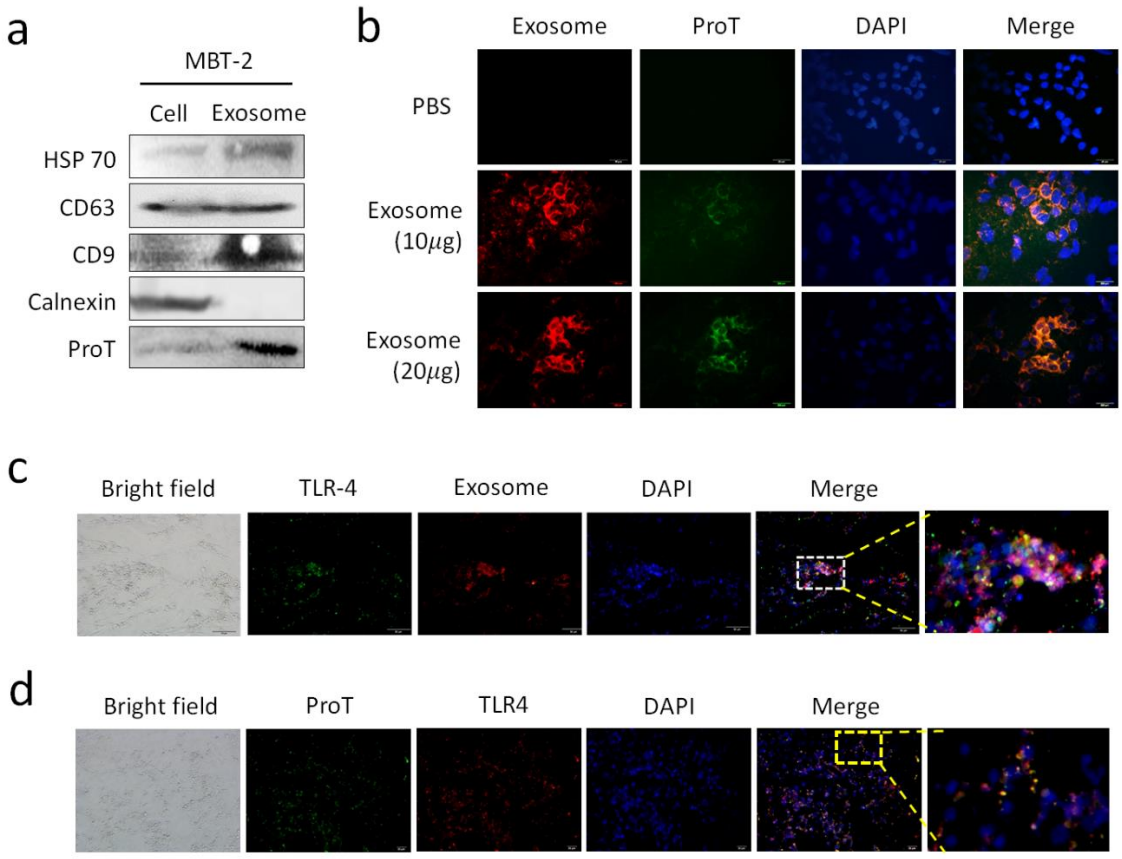


FIGURE 1 MBT-2 cancer cells derived exosomal ProT can bind TLR-4 in C2C12 cells. (a) Detection of HSP70, CD63, CD9, Calnexin and ProT expression in exosome by western blot. MBT-2 cells lysate was used as positive control with loading mass of 30 μg total proteins. Exosome represent MBT-2 derived exosome. (b) Characterization of DiI-labled MBT-2 cancer cells derived exosome(red) and ProT(green) at 2 h in C2C12 cells. Scale bar = 50 um. (c) Characterization of DiI-labled MBT-2 cancer cells derived exosome (red) and TLR-4 (green) at 2 h in C2C12 cells. Scale bar = 50 um. (d) Characterization of DiI-labled TLR-4 (red) and ProT (green) at 2 h in C2C12 cells. Scale bar = 50 um.

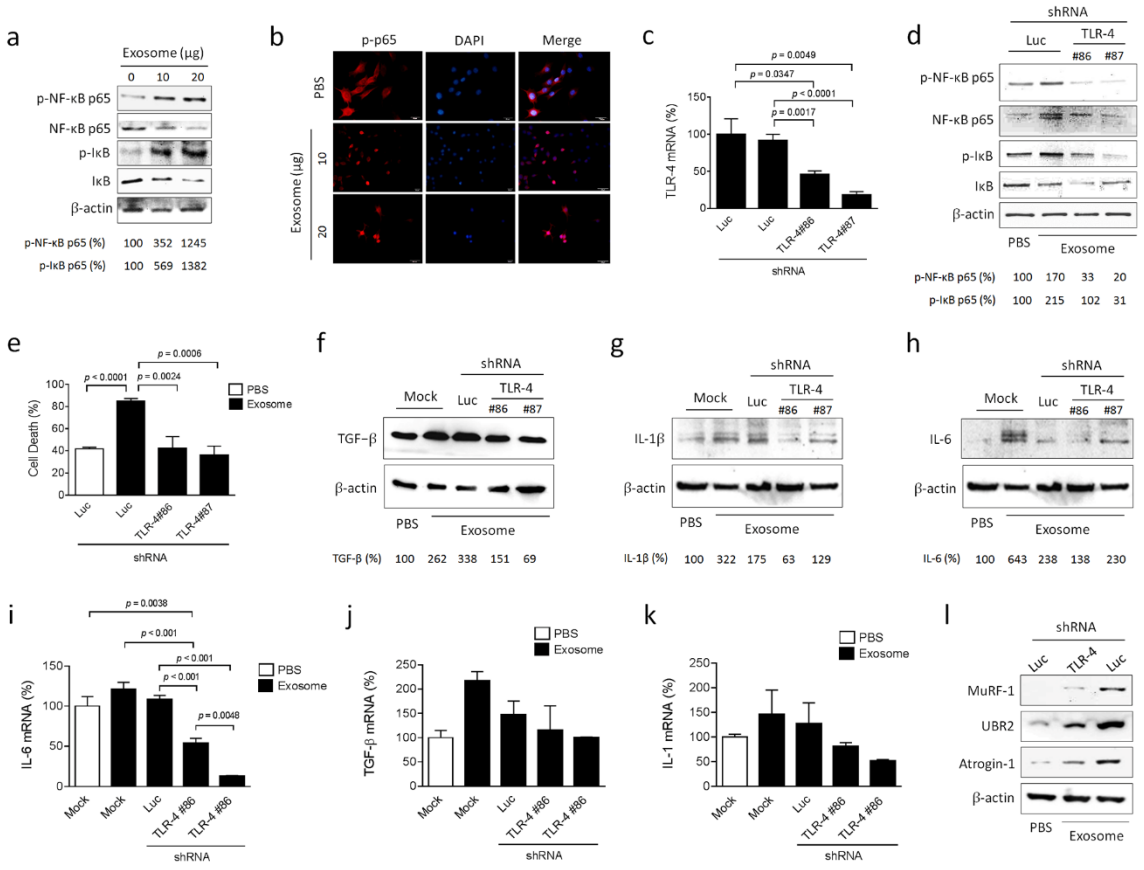


FIGURE 2 Exosomal ProT could activate NF-κB to translocate to the nucleus through TLR-4. (a) Activation of NF-κB in C2C12 was determined by western blot. The fold difference in band intensities was quantified and indicated under the image using the software of ImageJ. (b) Representative pictures of cellular immunofluorescence staining for p-p65 in C2C12 at 2 h. Immunocytochemistry staining was performed using an anti-p-p65 antibody (red) and DAPI (blue)for nuclear staining. (c) Relative mRNA expression of TLR-4 in C2C12 after knockdown by lentivirus were detected by RT-PCR. (d) C2C12 were pre-treatment with or without the shTLR-4(#86 and #87) and then incubated with MBT-2cell-derived exosome at 37 ˚C for 2 h. Activation of NF-κB in C2C12 was determined by western blot. The fold difference in band intensities was quantified and indicated under the image using the software of ImageJ. (e) Detect of C2C12 cell survival by MTS assay after treat exosome 2 h. (f) The protein expression changes of TGF-β, IL-1β, and IL-6 of C2C12 cells treated with MBT-2 derived exosomes. C2C12 cells were incubated with exosomes for 2h. (g) The mRNA level of TGF-β, IL-1β, and IL-6 at different treatment were detected by RT-PCR.

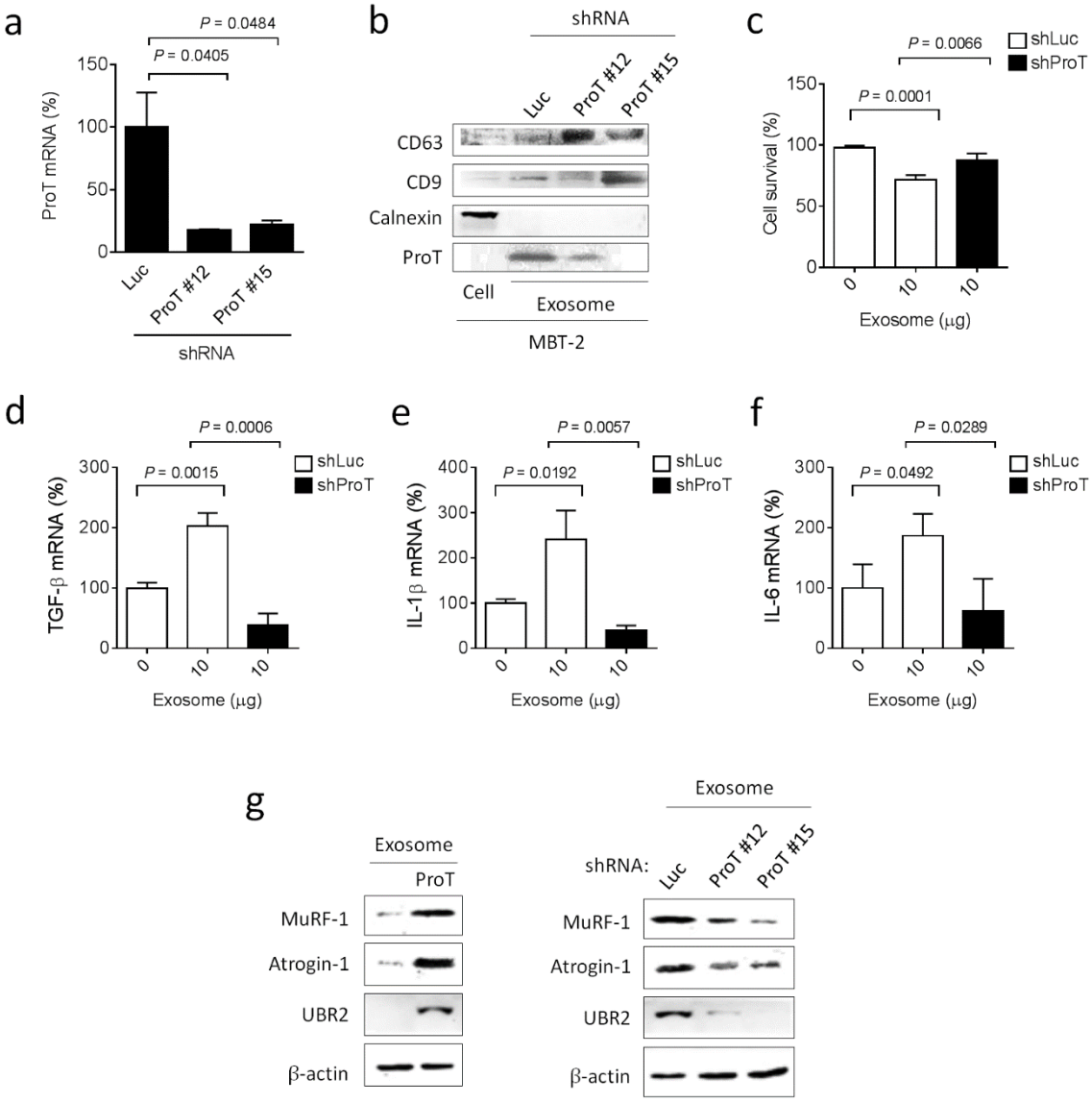


FIGURE 3 Exosomal ProT stimulate inflammatory cytokines production in C2C12 cells. (a) Knockdown ProT in MBT-2 cells by lentivirus. (shProT#12 and shProT#15). The mRNA expression changes of ProT were detected by RT-PCR after treatment with virus. (b) Characterization of exosomes secreted by MBT-2 (ProT knockdown) cells. (c) Detect of C2C12 cell survival by MTS assay after treat exosome 2 h. (d-f) The mRNA level of TGF-β, IL-1β, and IL-6 at different treatment (0=PBS, shProT= exosomes secreted by MBT-2(ProT knockdown) cells were detected by RT-PCR. (g) The protein expression changes of MuRF-1, atrogin-1 and UBR2 of C2C12 cells treated with MBT-2 derived exosomes. C2C12 cells were incubated with exosomes for 2h.

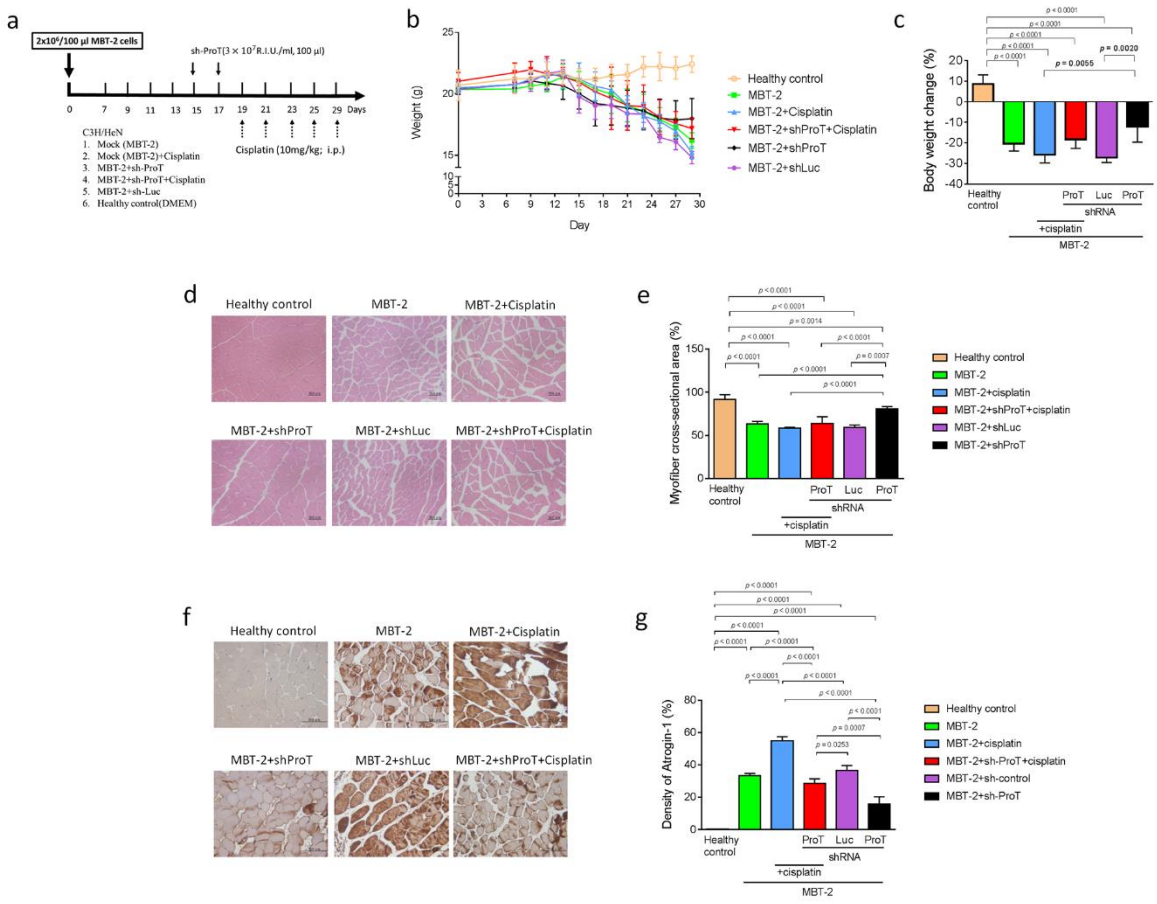


FIGURE 4 Knockdown of ProT alleviated the body weight decline in tumor-bearing mice. (a) For cachexia model generated by tumor cell, 100 μl MBT-2 cancer cells (2×10^6 cells), or an equal volume of vehicle (DMEM) was injected subcutaneously into the of 6-week old female C3H/HeN mice at day 0. Lentivirus sh-ProT were injected at day 15 and 17 (3×10^7 R.I.U./ml, 100ul) in group 3 and 4. Cisplatin was injected at day 19, 21, 23 , 25 and 29 (10mg/kg, i.p.) in group 2 and 4. (b) Bodyweight changes were recorded every two days after one week. (c) Final Bodyweight changes (%) were calculated and cartography by software of GraphPad Prism8. (d-f) After the mice were sacrificed, we separated the calf gastrocnemius muscle of the mice and observed it by H&E staining. Scale bar=50 μm. The myofiber cross-sectional area and number of myonucleus were calculated and recorded. (g) The protein expression of atrogin-1 were detected by IHC, Scale bar=50 μm. Density of atrogin-1 were calculated and cartography by software of GraphPad Prism8. (N=6)

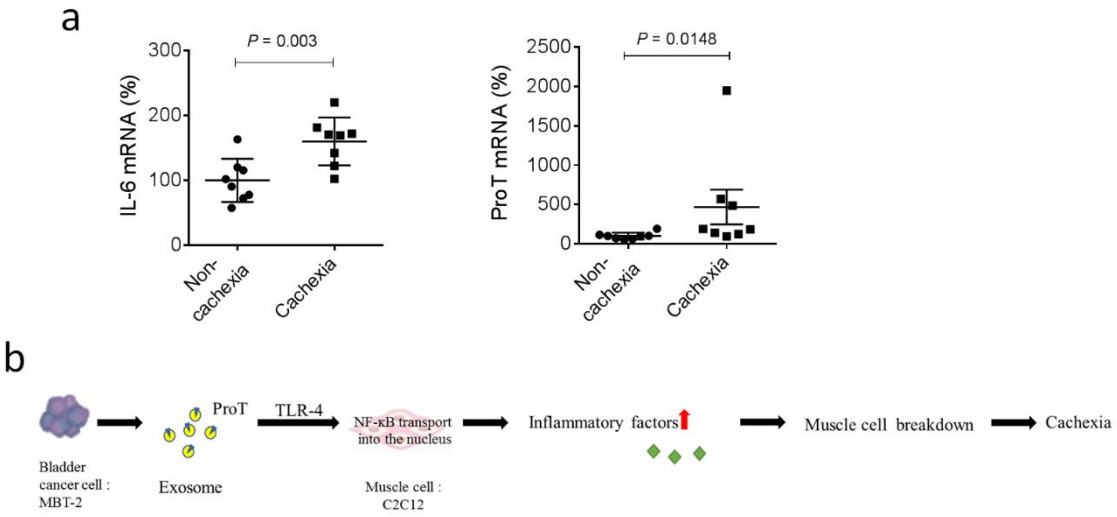


FIGURE 5 ProT may play an essential role in bladder cancer-associated cachexia. (a) The mRNA expression level of IL-6 and ProT in bladder cancer patients and in bladder cancer-associated cachexia patients ProT were detected by RT-PCR . (Mann-Whitney test, N = 8). (b) A schematic illustration showed that MBT-2 cell-derived exosomes affect C2C12 cells by activating NF-κB signaling through TLR-4 and result in cachexia.

主席提問：

在 Figure 1中，如何確認 TLR-4和癌細胞所產生的 exosome 具有直接作用？

答：只透過 Figure 1的免疫螢光染色確實無法證明兩者之間有交互作用，後續我們的實驗將使用共免疫沉澱（co-immunoprecipitation，co-IP）的方式來確認 exosome 中的 marker 像是 HSP70，CD63可能在 co-IP 中一起和 TLR-4一起被偵測到，來增加兩者之間存在直接或間接 interaction 的證據。

在 Figure 2中，只測定 cytokines 的 mRNA，是否缺乏直接證據 cytokine 的表現真的會增加？

答：我們將使用 ELISA 的方式來測定真實的 cytokines 的表現量。

聽眾提問：

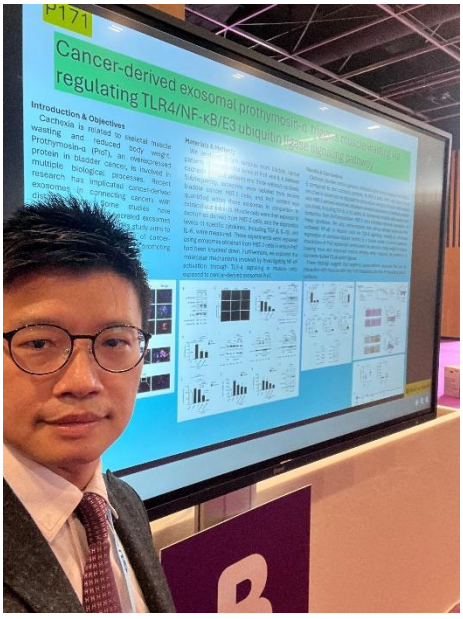
我們如何分離出 exosome？

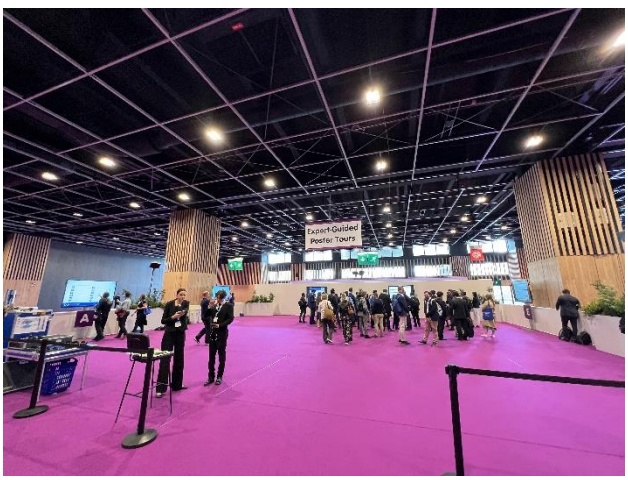
答：我們使用高速離心的方式。我們將 MBT-2細胞在無血清之 DMEM 中培養16小時。然後，收集培養基並以300g 離心30分鐘，以去除細胞，接著以3000g 離心30分鐘，再以額外的10,000g 離心1小時。最後，將上清液和16% PEG 溶液以1:1的比例混合。然後，上清液在4°C 下使用 SW28離心機以100,000g 的速度超速離心1.5小時。得到的沉澱物在 PBS 中再次在4°C 下以100,000g 的速度超速離心1.5小時。沉澱物將置於新的 PBS 中。

如何證實我們所得為 exosome？

答：我們使用 HSP70、CD63和 CD9這些標記蛋白来確認所得物質是否為 exosome。

心得及建議：





本次的報告方式很特別，是站在可互動式的海報前面報告。但不同於傳統的海報由我們「被動」站在海報前等人前來觀賞及發問，有可能會有人乾脆沒來貼海報這種情形，這次每個 session 會有兩位 moderator，在離觀眾很近的大螢幕前「主動」播放你的海報，讓你先簡報後再提問，並讓台下觀眾發言及提問。這很大程度拉近了講者和觀眾的距離，讓主題的呈現更像是一種「小組討論」的方式，不像上台演講一樣距離觀眾很遠，我覺得這種方式可能將來會越來越盛行，也可做為國內在年會或類似會議上的一種參考模式。只是大家都被迫站著大概久了會覺得有會累，所以每個 session 可能時間不能太長。