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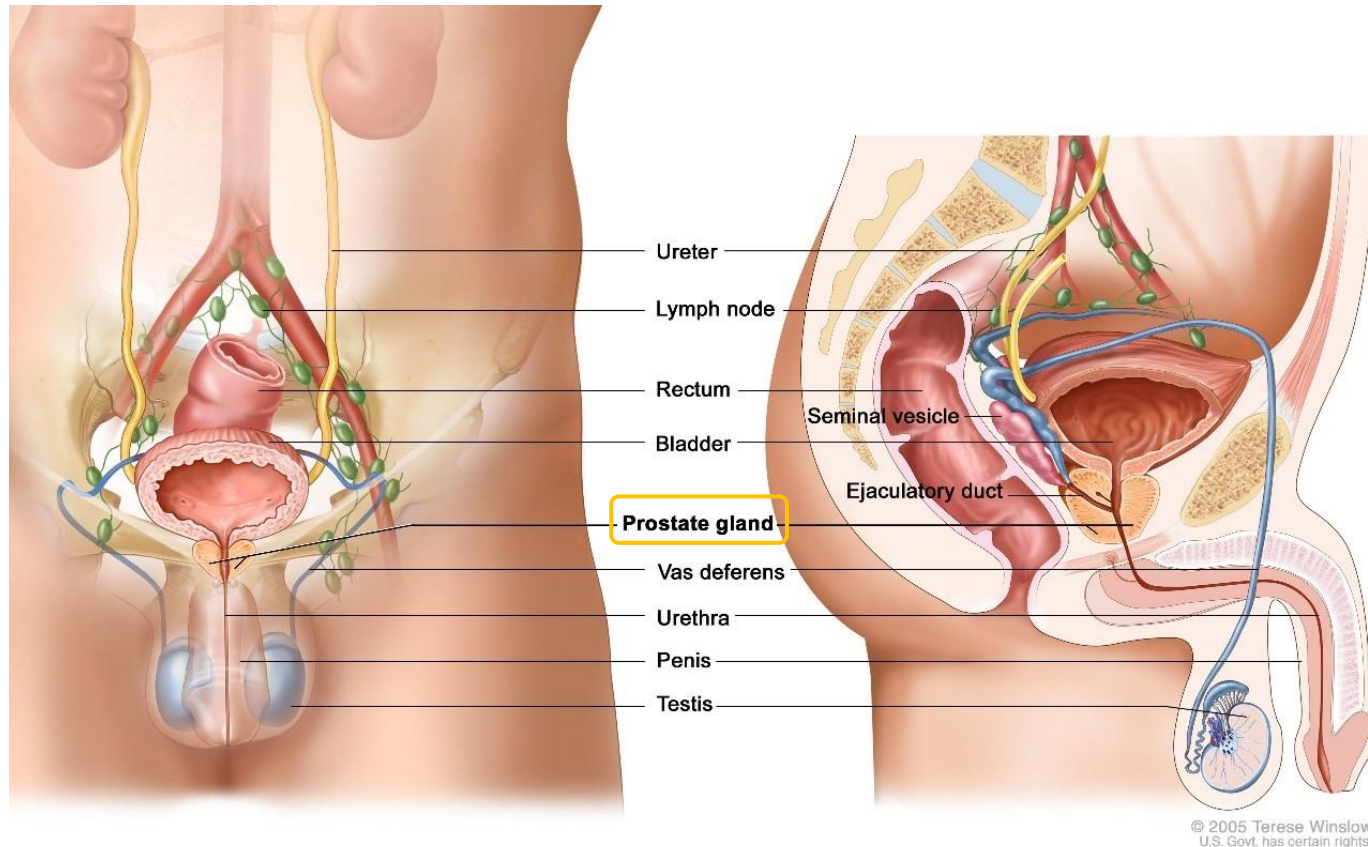
Exosomal matrix metalloproteinases 2 and 9 in Prostate Cancer

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Prostate Cancer (PCa)



2nd most frequent type
of cancer in men

Heterogeneous progression
and differentiation

Current biomarker: PSA, poor
specificity

Diagnosis based on histological
characteristics (Gleason Score)

Figure 1. Winslow, T. (2005). *Anatomy of the male reproductive and urinary systems*. Available at: <https://www.cancer.gov/types/prostate/hp/prostate-treatment-pdq> [Consulted 28-3-2021]

Gleason Score

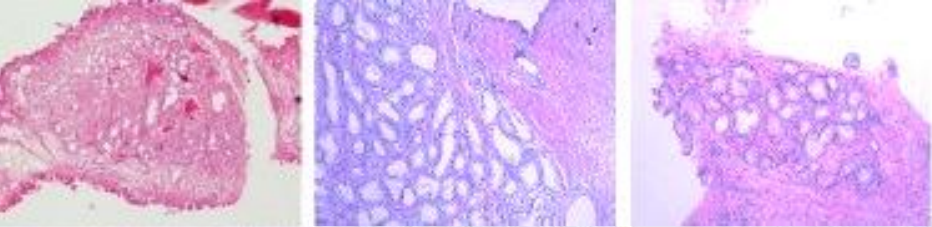
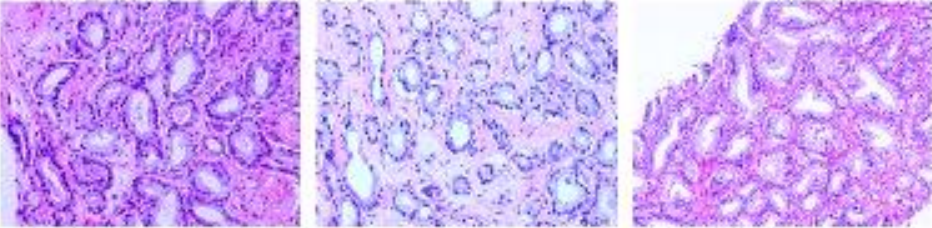
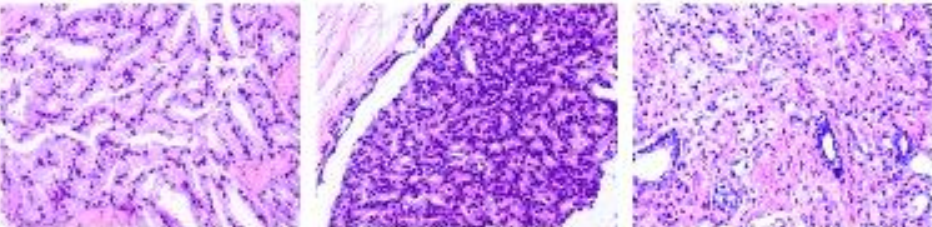
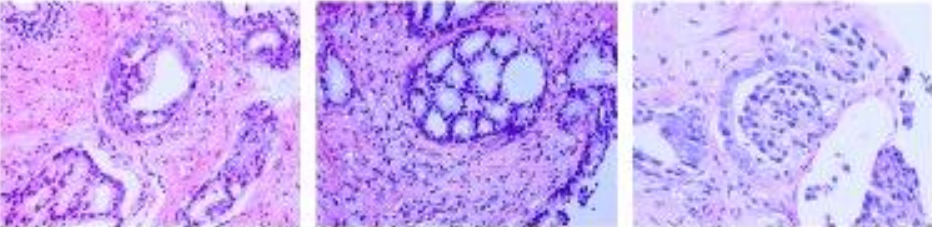
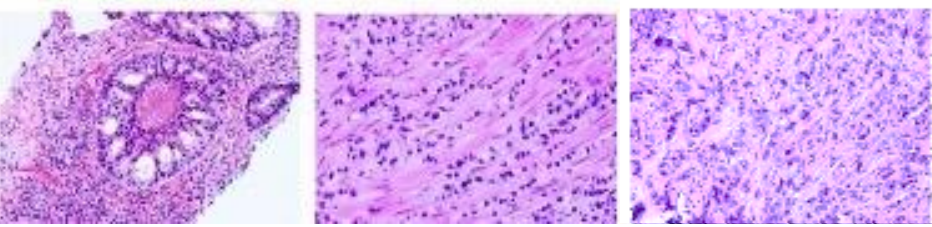
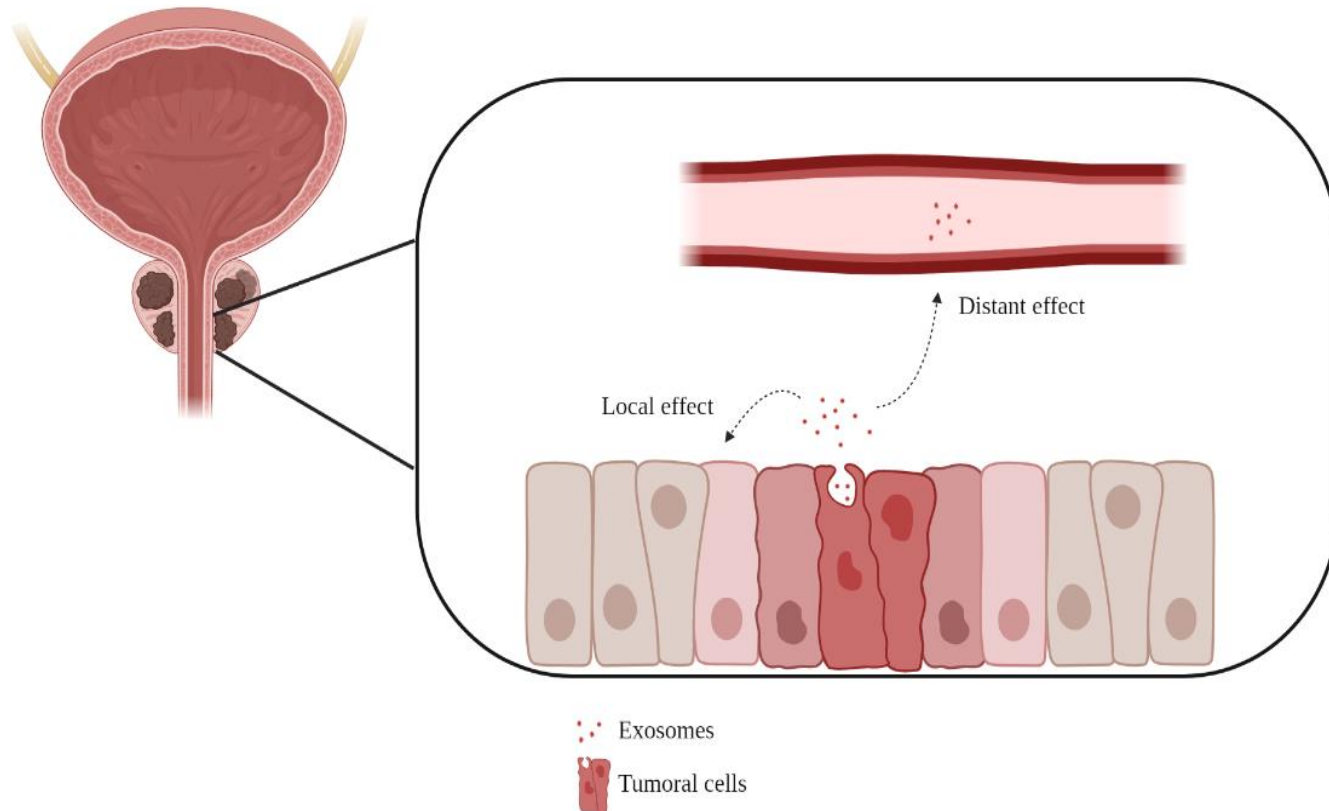
	Gleason patterns 1-3 distinct, discrete, individual glands	Gleason score ≤ 6	Grade group I
	Gleason pattern 4 fused, cribriform, or poorly-formed glands, or glomerular	Gleason score $3+4=7$	Grade group II
		Gleason score $4+3=7$	Grade group III
		Gleason score $4+4=8$ $3+5=8$ $5+3=8$	Grade group IV
	Gleason pattern 5 comedo necrosis, cords, sheets, solid nests, single cells	Gleason score $4+5=9$ $5+4=9$ $5+5=10$	Grade group V

Figure 2. Gleason patterns. Chen, N., & Zhou, Q. (2016). *The evolving Gleason grading system*. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4779758/> [Consulted 28/3/2021]

Exosomes and MMPs in PCa



Exosomes

- Extracellular vesicles (\varnothing 30-150 nm)
- Found in human fluids (blood, urine)
- Implied in cell communication

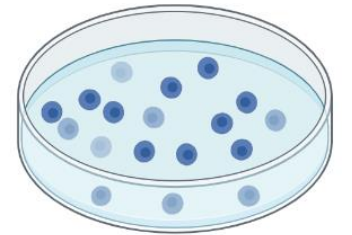
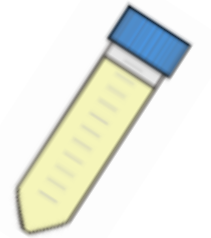
MMPs

- Carried by exosomes
- Involved in epithelial mesenchymal transition (EMT)
- Related to a more aggressive phenotype of PCa

Figure 3. Role of exosomes and matrix metalloproteinases (MMPs) in PCa. Huertas, R. (2021). Created in BioRender.com

Hypothesis

- Exosomal matrix metalloproteinases 2 and 9 could be used as a biomarker in liquid biopsy (urine) in prostate cancer.
- The study of liquid biopsies will be complemented by the immunohistochemistry analysis of needle prostate biopsies.
- Finally, we will study the effect of isolated exosomes from urine on the neurodifferentiation of androgen-dependent prostate cancer cell line (LNCaP).



A decorative graphic consisting of several concentric, overlapping curved lines in shades of blue and green, forming a partial circular shape around the central text.

Methods

Study sample

	Non cancer	ASAP	Low risk (Gleason 6)	Intermediate risk (Gleason 7)	High risk (Gleason < 8)
N	18	7	7	12	9
Age	66 ± 5.5	64 ± 5.4	67 ± 5.5	68 ± 7.7	66 ± 11.1
PSA levels	7.35 ± 3.89	8.63 ± 6.01	9.92 ± 11.24	11.82 ± 6.91	613 ± 1367.09

- Urine and needle biopsies samples were provided by the Urology Service of University Hospital Príncipe de Asturias.
- Urine samples were collected before prostate stimulation (pre-prostate massage) and after (post-prostate massage).
- LNCaP cells (androgen-dependent PCa cell line) were used to analyze the effect of treatment with exosomes isolated from urine samples.

Exosomes isolation

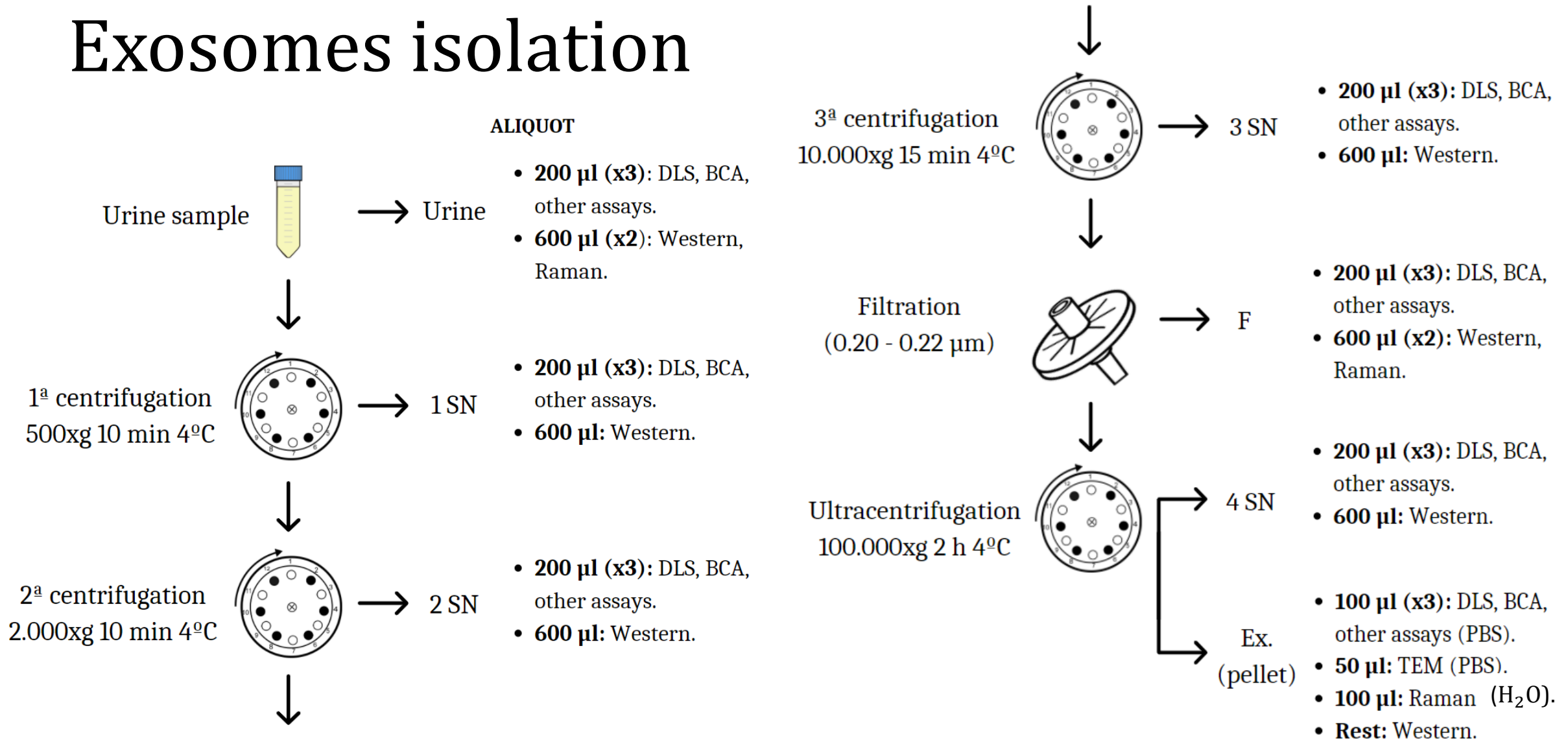


Figure 4. Exosomes isolation. Huertas, R. (2021). Supernatant (SN) was collected after each centrifugation. Exosomes are found in the pellet of the ultracentrifugation. Created in Canva.com

Biochemical assays

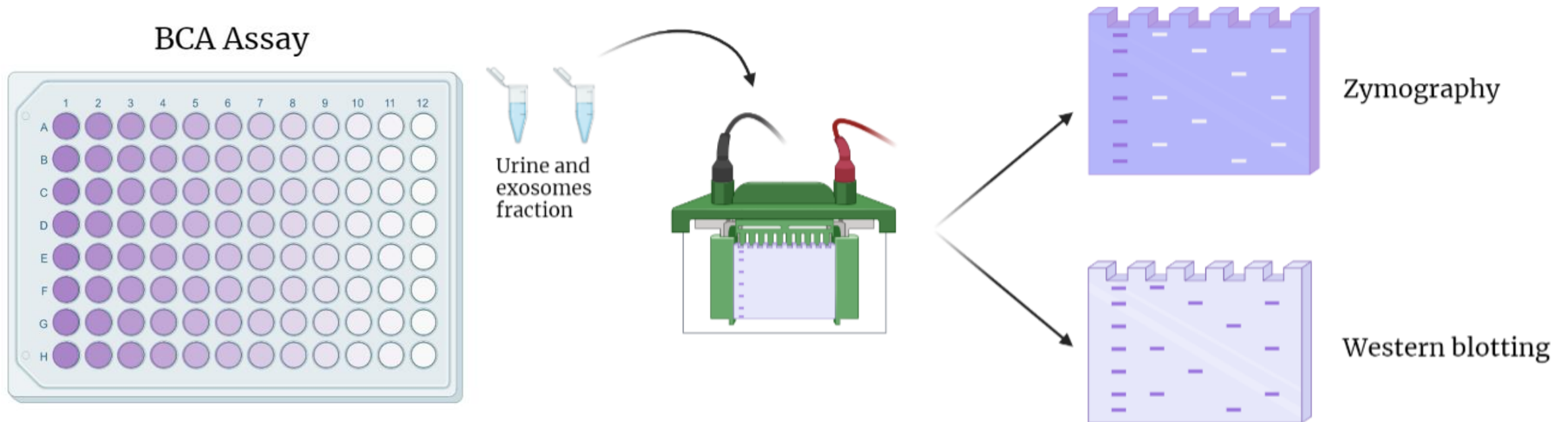
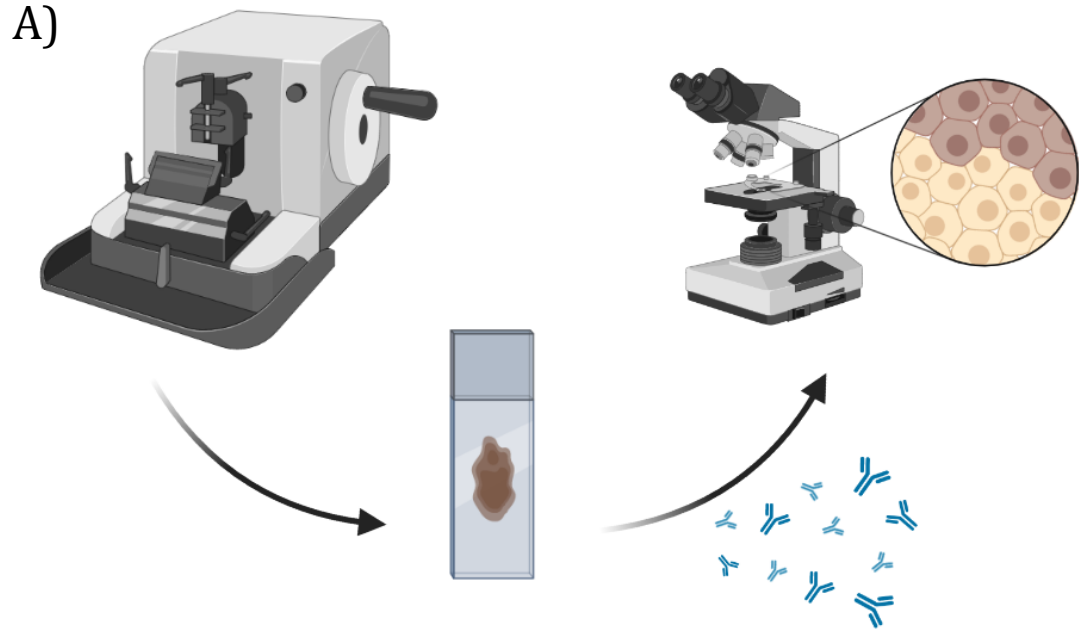


Figure 5. Biochemical assays. Protein concentration of each sample was determined by BCA assay using a commercial kit [BCA Protein Assay Pierce™ (Thermo Fisher)]. Then, zymography was used to study MMPs activity, by adding porcine gelatin to the lower gel, which is degraded by MMPs 2 and 9. Gelatin degradation leaves a white band in the gel after staining and destaining, showing MMPs migration and activity. Finally, MMPs levels will be analysed by Western blotting.

Immunohistochemistry



Cell cultures

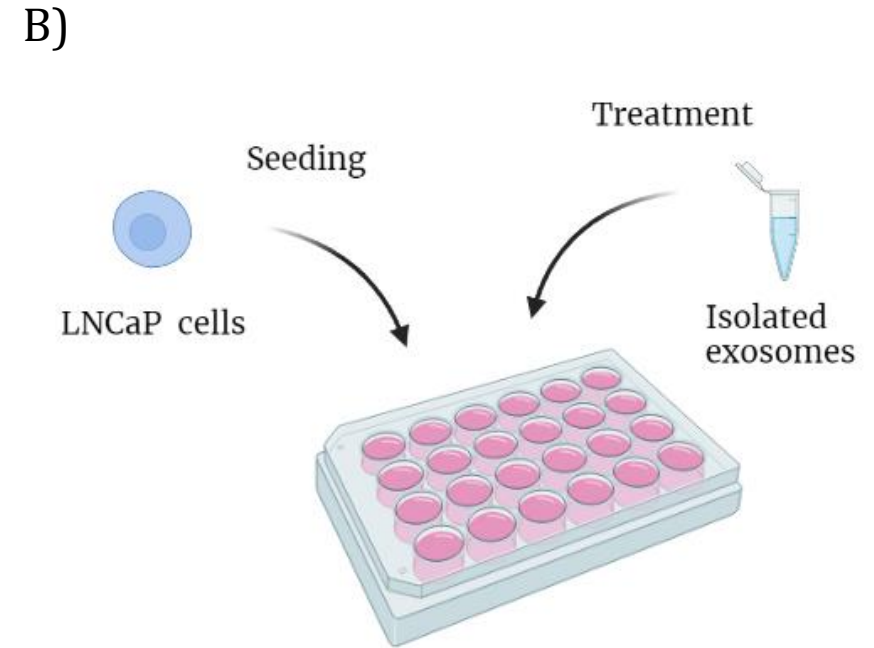


Figure 6. A) Histological expression of different molecules was examined by immunohistochemistry. B) Effect of exosomes isolated from liquid biopsies on LNCaP cells.

The background features a large, stylized graphic composed of multiple overlapping, semi-transparent curved bands. The left side of the graphic is primarily light blue, while the right side transitions into light green. The bands are arranged in a way that creates a sense of depth and movement, framing the central text.

Preliminary results

Zymography

Urine Exosomes

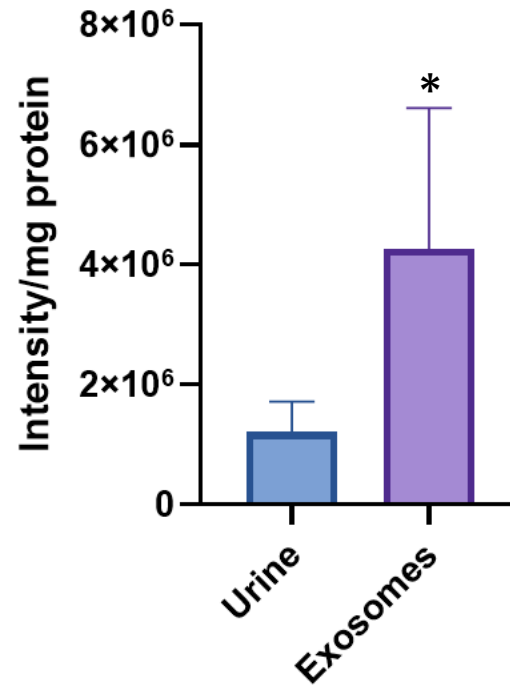
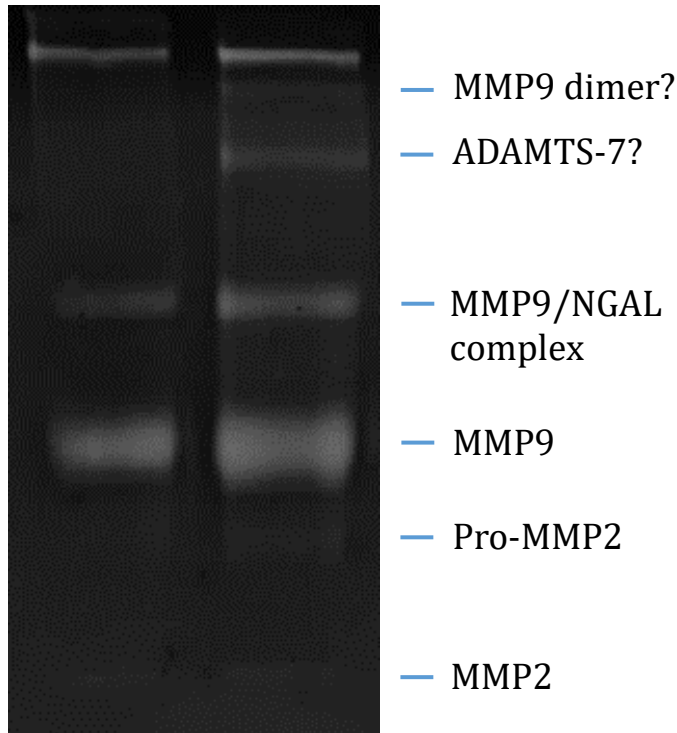


Figure 7. Signal purification. Bands intensity/mg protein ratio from post-prostate massage urine and isolated exosomes were compared. Exosomes showed a statistically significant increase (n=22 ; *, $p < 0.05$).

Next steps

- Study the gelatinase activity of the remaining post-prostate massage samples.
- Analyse bands patterns between comparison groups with different Gleason score.
- Study pre-prostate massage urine samples.

Immunohistochemistry

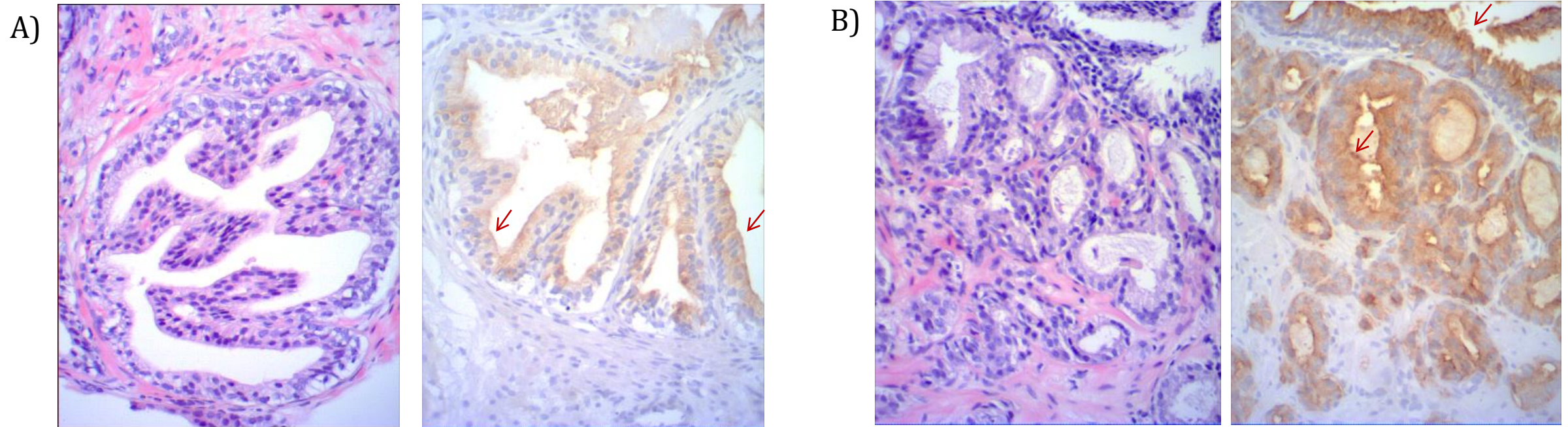


Figure 8. Needle prostate biopsy 5 μm cross-section. Left: H&E stain, 20X. Right: IHC using PSMA (prostate specific membrane antigen) rabbit antibody, 20X. A) Non cancer sample. B) Gleason 6 sample. PSMA is located in the apical cytoplasm and membrane of the epithelial cells (red arrows). PSMA signal is more intense in Gleason 6 samples than in non-cancer samples.

Next steps:

- Study the expression of other molecules, such as MMP2 or MMP9.
- Analyse intermediate and high risk needle biopsies.

Neuroendocrine differentiation

Neuroendocrine cells constitute just 1% of the epithelial cells in normal prostate tissue. In PCa, the increment of neuroendocrine-differentiated (NED) areas has been associated with tumour progression and castration-resistant cancer.

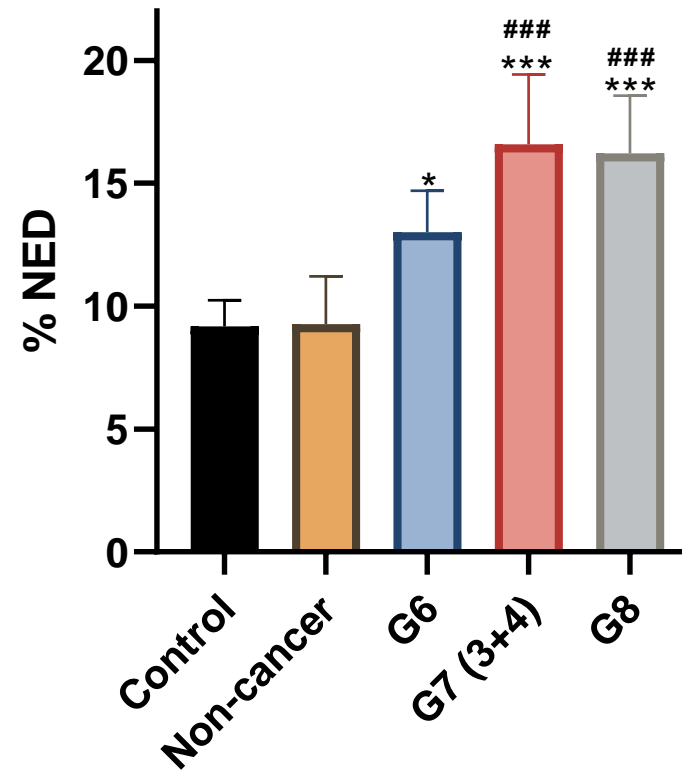
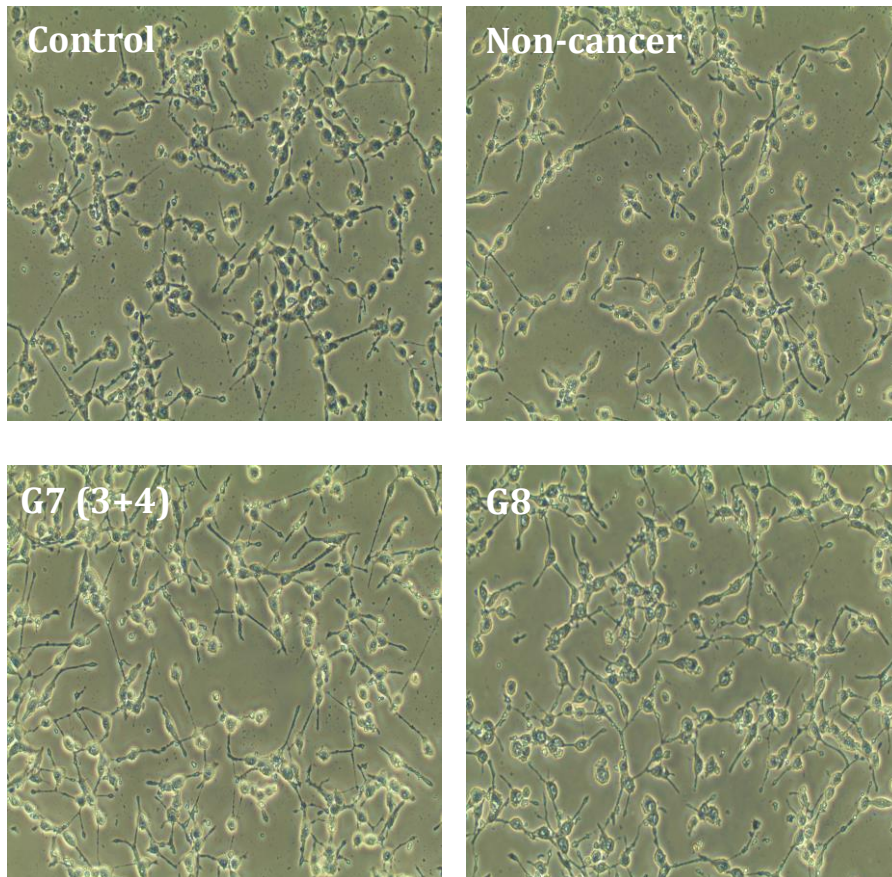


Figure 9. NE differentiation of LNCaP cells 24h after treatment with exosomes of PCa patients. Preliminary results showed a significant increment of neuroendocrine phenotype in LNCaP cells treated with exosomes of PCa patients. *, $p < 0.05$; ***, $p < 0.001$, with regard to control (untreated cells); ###, $p < 0.001$, with regard to cells treated with exosomes of non-cancer patients.