In vitro culture and characterization of circulating tumor cells from patients with urothelial carcinoma

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INTRODUCTION

Circulating tumor cells (CTCs)

- A new promising biomarker for predicting prognosis and monitoring therapeutic response.
- Detected in most advanced epithelial cancers including lung, prostate, pancreatic, colon, breast , and bladder cancers.
- Variable CTC capture method
  - Density centrifugation
  - Immunomagnetic separation
  - Filtration (isolation by size)

Cytogen's CTC enrichment platform

- Size-based filtration
- High Density Microporous (HDM) chip
- Filtration without pressure (damage-free recovery of live CTCs)

The purpose of this study

- To evaluate the feasibility of isolation and culturing of circulating tumor cells (CTCs) for the as an alternative to tumor tissue biopsy in patients with urothelial carcinoma.

MATERIALS AND METHODS

Blood collection

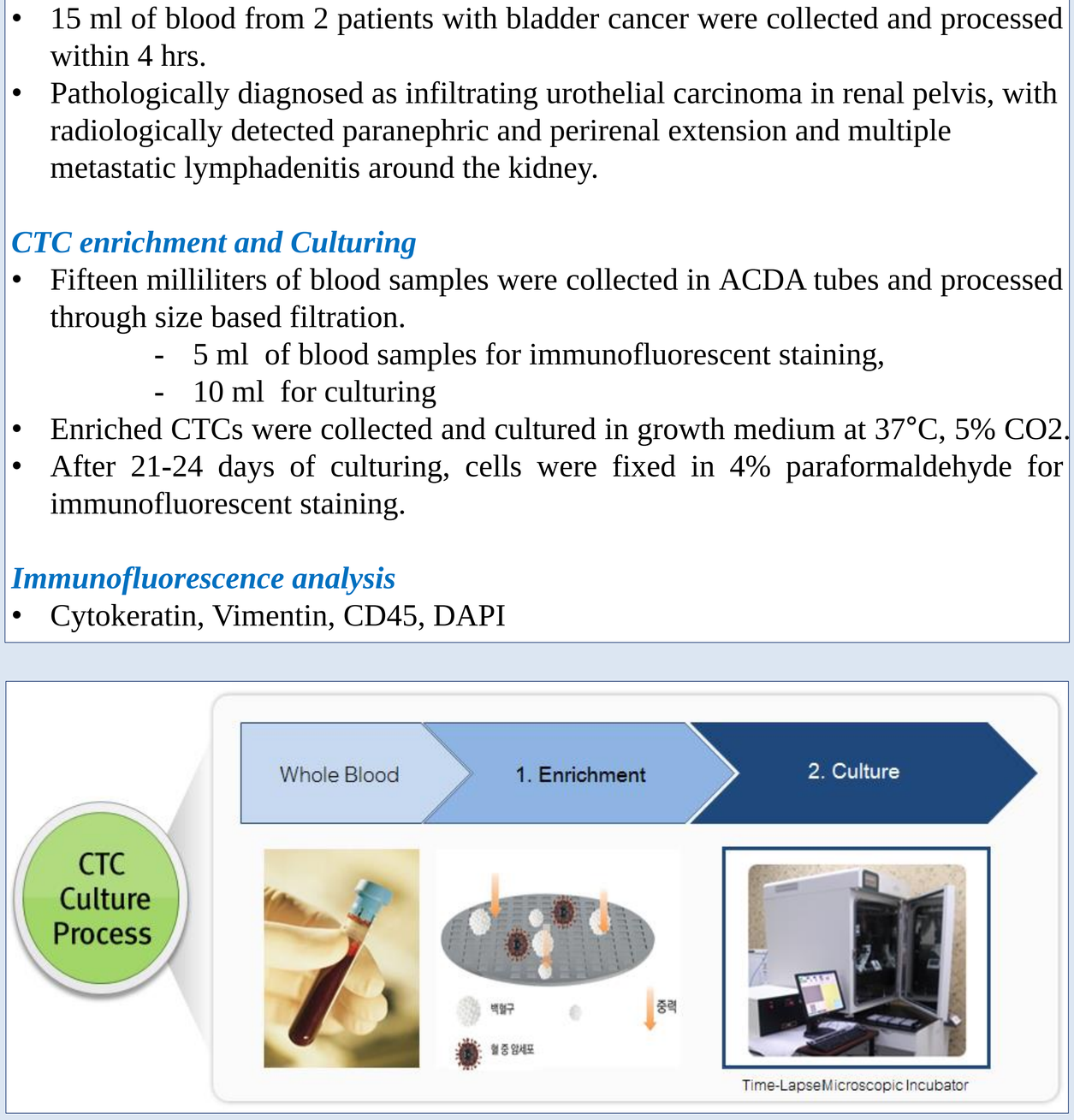
- 15 ml of blood from 2 patients with bladder cancer were collected and processed within 4 hrs.
- Pathologically diagnosed as infiltrating urothelial carcinoma in renal pelvis, with radiologically detected paranephric and perirenal extension and multiple metastatic lymphadenitis around the kidney.

CTC enrichment and Culturing

- Fifteen milliliters of blood samples were collected in ACDA tubes and processed through size based filtration.
  - 5 ml of blood samples for immunofluorescent staining,
  - 10 ml for culturing
- Enriched CTCs were collected and cultured in growth medium at 37°C, 5% CO2.
- After 21-24 days of culturing, cells were fixed in 4% paraformaldehyde for immunofluorescent staining.

Immunofluorescence analysis

- Cytokeratin, Vimentin, CD45, DAPI

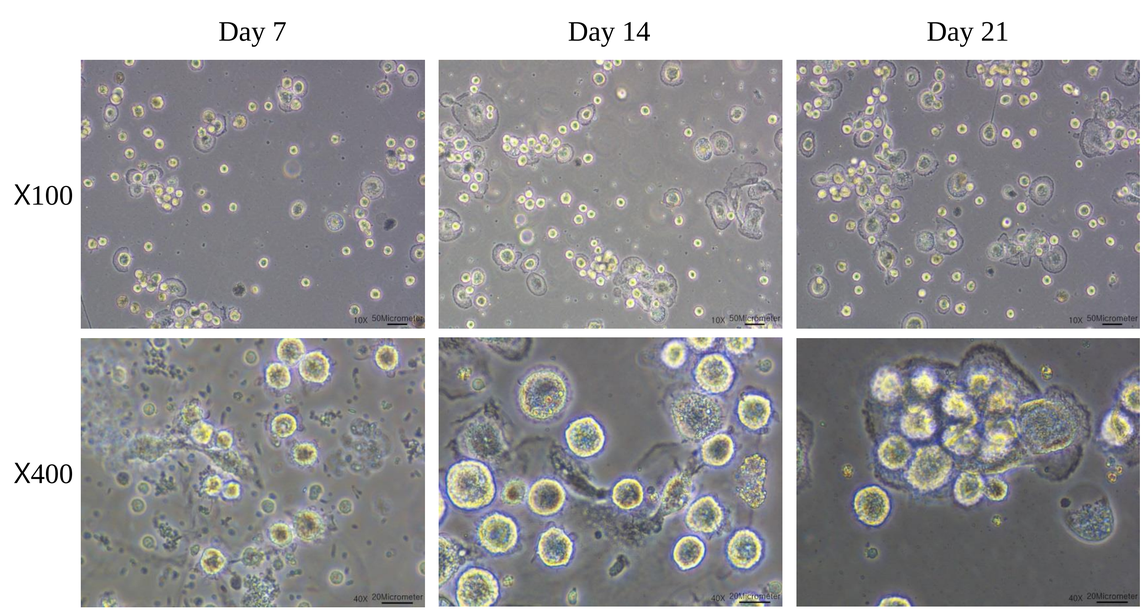


RESULTS

Representative images of CTCs

CTC count	DAPI stain	Vimentin stain	CK stain	CD 45 stain	Merged finding
#1					
#2					
#3					
WBC					
Vimentin, CK (+) control					
CD45 (+) control					

Representative Microscopic images of cultured CTCs at day 7, 14, and 21



CONCLUSION

1. Here we isolated and successfully cultured CTCs from patients with bladder cancer.
2. These results suggest that the isolation and culture of CTCs can be an effective method to obtain relatively large number of cells.
3. Further studies with large number of samples are needed to verify the clinical utility of CTC analysis.