INTRODUCTION

Renal Cell Carcinoma (RCC) is the deadliest urologic cancer. It is the 7th most common cancer in men and the 10th in women. It is usually diagnosed at late stage during routine check-ups and surgery remains the most effective curative method. RCC occurs mainly in 60-70 years old men and the rate of diagnosis is increasing due to greater use of advanced imaging. RCC tissues show a considerable heterogeneity tumour microenvironment (TME) which can differ from patient to patient and within the same patient. Characterizing the heterogeneity of the TME and its association with progression is crucial for better understanding this disease. New imaging and molecular biology techniques can improve stratification of patients risk of recurrence and metastasis, in order to better predict RCC prognosis and allow personalized therapy. Here we present a combination of two complementary techniques in order to identify novel prognostic markers able to better predict RCC patients at a high risk of metastasis, opening the door to personalized therapy.

OVERALL GOALS

1. Compare differentially expressed proteins for association with:
   - Risk of metastatic disease;
   - Cancer-related survival.

2. Use multiplexed immunofluorescence (IF) and image analysis to automatically segment the tumour and stroma regions of interest (ROI) on a RCC TMA and quantify lymphocyte and immune marker co-localization.

3. Use NanoString® GeoMx™ Digital Spatial Profiling (DSP) Technology to better understand the drivers behind tumour progression within matched patient samples at various disease progression states and linkings profiles to patient outcome.

ADVANCED IMAGE ANALYSIS (AIA)

MATERIALS & METHODS

- 177 patients having primary, renal or inferior vena cava thrombus (VIT) or distant metastasis were collected on 11 TMA slides. 4-genes IF was performed, where CD3, PD1, FO-D1-1 and an epithelial marker consisting of a pan-cadherin CAIX/CD105 was used. Definiens Tissue studio was used to perform tumour/stroma segmentation and immune marker co-registration and quantification. PD-1 was excluded from the results because of too low signal-to-noise ratios.

- One TMA was selected among the 11 and a serial section after IF taken for DSP. From this slide, 78 cores were selected, representing a minimum of 3 normal, primary tumour, VIT and metastatic cores for each patient. A total of 12 patient samples were analysed by DSP.

- A 45 antibody panel, including IgG quality controls was used with NanoString GeoMx DSP Cores were probed with an IF on 50-100um thick section of the core. Markers having an expression level lower than the IgG controls were excluded.

WORKFLOW

- Advanced image analy- sis: green cancer cells; red CD3 positive cells; blue nucleus; A/Immunofluorescence localization: image analysis; tissue analysis; tissue analysis

- Identification and 4-gene classification in the DSP slide

- Digital barcoding is possible without disrupting the samples. A spatially resolved, highly multiplexed (up to 800 proteins) strategy could become an essential tool to discover novel prognostic biological markers in a variety of diseases.

ADVANCED IMAGE ANALYSIS RESULTS

- Normal cores showed wide variance compared to tissue with high-DGS.

- Markers with high-DGS showed increased expression compared to tissue with low-DGS.

- Performing AIa on normal TMAs

- Performing tumour/stroma segmentation on the DSP instrument

- Combine DSP and Image analysis results

- Run this strategy across the whole cohort (177 patients)

- Use a "sandwich" strategy in order to combine two TMA panels to DSP

ANALYSIS OF IDENTIFIED MARKERS

- 1. Serial TMA slides stained with different outcome
- 2. Deparaffinization and de-waxing
- 3. Antigen retrieval
- 4. Endogenous peroxidase block
- 5. Blocking serum
- 6. Antibody incubation
- 7. Secondary antibodies
- 8. DAB detection
- 9. Hematoxylin counterstaining
- 10. Slides are dehydrated and coverslipped

DIGITAL SPATIAL PROFILING RESULTS

- Performing AIA on normal TMAs

- Performing tumour/stroma segmentation on the DSP instrument

- Combine DSP and Image analysis results

- Run this strategy across the whole cohort (177 patients)

- Use a "sandwich" strategy in order to combine two TMA panels to DSP

CONCLUSIONS

- AIA allowed the segmentation of tumour and stroma compartments and the analysis at single cell resolution. This demonstrated the correlation of CD3 and PD1 with disease-specific risk and rate of metastases from analyzing the primary ccRCC TMAs.

- DSP allowed highly-paired protein profiling of the cores. This demonstrated the differences in the protein patterns observed in normal, primary and VIT ccRCC tissue from patients with different outcomes are associated with variable survival and risk of metastases.

This work demonstrates the feasibility and applicability of combining advanced image analysis and with multiplexed digital spatial profiling for a better risk stratification of patients. The combination of these strategies could become an essential tool to discover novel prognostic biological markers in a variety of diseases.

FUTURE PLANS

- Performing AIa on normal TMAs

- Performing tumour/stroma segmentation on the DSP instrument

- Combine DSP and Image analysis results

- Run this strategy across the whole cohort (177 patients)

- Use a "sandwich" strategy in order to combine two TMA panels to DSP

REFERENCES


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