CReMEC initiative: Characterization of patient-derived colorectal tumor models and correlation with patient profile

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INTRODUCTION

Well characterized models representing the heterogeneity of human colorectal cancers (CRC) are needed to develop effective therapeutic agents. Establishment of such tools will allow a better prediction of the clinical outcome, taking into account the diversity of each patient tumor phenotype and genotype. For this purpose, we have associated efforts from hospitals, academic groups, biotech and pharmaceutical companies. The goal of this consortium is to create an experimental tumor model resource center to improve or strengthen drug development. From May 2007 to January 2010, 88 surgical specimens (58 primary, 15 metastases, 60 peritoneal carcinomatosis (PC) were collected from CRC patients (with informed consent and negative HBV, HCV, and HIV serologies). Tumor samples were subsequently xenografted in nude mice and characterized as described below. We report here the results on our panel of models.

MATERIAL AND METHODS

Clinical data collection

- Retrospective clinical data were collected by the attending physician and included in a standardized data sheet.
- Identification of the data sheet is anonymous.

Histological characterization

- Samples were fixed for a maximum of 48 hours in alcohol-formalin-acetic acid (AFA) and embedded in paraffin. 5 µm sections were stained with hematoxylin-eosin.
- Molecular characterization

- CGH analysis showed very similar profile between early and advance passages
- The following genetic markers, relevant in CRC, were selected for sequencing:
  - APC (exons 2 & 3), CTNNB1 (exons 9 & 16), PIK3CA
  - MSI-L
  - Other

- DNA sequence analysis and Sanger sequencing was carried out in 16 tumor samples, within 15 hour after specimen collection. Mutations were performed according to well-established guidelines for clinical care and handling.
- Genomic DNA from tumors were sequenced subcutaneously in nude mice and compared to the original samples in the corresponding CRC xenograft.

- EGFR mutated pathway
- Genotypes of CRC were analyzed by tumor xenografts to assess the degree of sensitization to EGFR-targeting therapies.
- The expression and localization of EGFR were assessed by immunohistochemistry and western blot analysis. The expression of the specic targets was confirmed by real-time polymerase chain reaction.

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  - APC (exons 2 & 3), CTNNB1 (exons 9 & 16), PIK3CA
  - MSI-L
  - Other
- Preservation of the tumor phenotype and genotype

- Clinical and pathological parameters were recorded and compared to the original clinical data.
- The majority of tumor models were negative xenografts, which are less relevant to predict outcomes.

- Most models with EGFR mutated pathway are resistant to Cetuximab, whereas several Cetuximab-sensitive KRAS-mutated models have been identified.

- Diversity of colorectal cancers is fully addressed in this collection of patient-derived tumor models.
- Genotype and phenotype are largely preserved throughout the model establishment process.
- Difference of gene mutation and drug sensitivity profiles are observed between the models.
- Plan to complete the full correlation analysis between clinical data, gene mutations, transcriptome profile, ex-vivo and in vivo drug sensitivity.
- Perspectives to fully exploit this new collection for new drug candidate selection.

RESULTS

Clinical characteristics and in vivo tumour graft take rate

- Significant distribution of primary tumors in regard of lymph node status (panel A, N1 = 1 to 6, N2 = 7 to 15 positive regional lymph nodes), stages (panel B) and differentiation status (panel C).
- No other significant correlation were found among the following parameters: gender, age, resection extent, lymphatic embolies, perinervous invasion, initial treatment, genotype

- Patient characteristics for the collection

- Preservation of the tumor phenotype and genotype

- Only 1 model showed a poor differentiated (PD) to undifferentiated (UD) characteristic, as well as its correspondent patient

- No other significant correlation were found among the following parameters: gender, age, resection extent, lymphatic embolies, perinervous invasion, initial treatment, genotype

- Elective chemotherapy was applied to 5/8 patients, with response rates varying from 40% to 100%.

- Multiple mutated profiles are observed in our tumor panel (n=9), matching the human tumor genetic heterogeneity

- Tumor xenografts exhibit diversity in the response to chemotherapy

- 13/8 tumour xenografts show regression after treatment with at least one standard chemotherapy

- Similar tumor responses have been observed in mice and rats

- Gene mutation profile and response to chemotherapy

- Percentage (%)

- Sensitivity to Cetuximab versus mutated genes involved in EGFR pathway

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CONCLUSION

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