



ILCA21 Molecular Pathogenesis, Cell Biology and Translational Research

TRANSCRIPT

00:06 --> 00:23 - Amaia Lujambio

Hello everybody, my name is Amaia Lujambio and I'm an Associate Professor at Mount Sinai in NY working on mouse model of hepatocellular carcinoma. And I'm here with Stephanie Roessler from Heidelberg to discuss the General Session 1 on Molecular Pathogenesis.

00:24 --> 00:46 - Stephanie Rössler

Hello everyone, my name is Stephanie Roessler and I am a research group leader at Heidelberg University Hospital in Germany. My research focusses molecular analyses of hepatocellular carcinoma and cholangiocarcinoma patient cohorts.

00:46 --> 01:05 - Stephanie Rössler

The first talk of the session was by Dr. Philip Haber from Mount Sinai in NY on "Molecular Markers of Response to anti-PD-1 therapy in advanced hepatocellular carcinoma". So, Amaia, what did you think were the main highlights of this talk?

01:05 --> 01:24 - Amaia Lujambio

This is a very relevant and timely work given the importance of immunotherapies based on immune checkpoint inhibitors in hepatocellular carcinoma right now. As you know, nivolumab and pembrolizumab, which are the anti PD1 inhibitors, are both approved in second line therapy.

01:24 --> 01:43 - Amaia Lujambio

However, it is still not clear which patients are more likely to respond to immunotherapies. So in this study, what they did is they took samples from advanced HCC patients. And this was a huge collaborative effort. They got samples from 13 different centers in seven different countries.

01:44 --> 01:58 - Amaia Lujambio

And they did a microarray of different characterization of the samples by performing transcriptomic analysis, sequencing the CTNNB1 gene and also performing immunohistochemistry.

01:59 --> 02:51 - Amaia Lujambio

As expected, responders had a significantly longer survival than non-responders so that demonstrates that they chose well how to call every patient. In addition, those patients receiving anti-PD-1 as frontline therapy were more likely to respond than those that receive it as second line therapy. In those patients, response to anti-PD-1 was associated with IFNG signaling, MHC-

class 2 formation, and MHC-class 2-dependent antigen presentation. So one of the findings that I found more interesting was that CTNNB1 mutation was not linked to response to anti PD1.

02:52 --> 03:11 - Amaia Lujambio

A previous study in HCC patients from Memorial Sloan Kettering had seen enrichment of patients with CTNNB1 mutation in the resistant group. And a study from my own group has shown that in mouse models, beta-catenin mutation is associated with resistance to immunotherapy in mouse models.

03:12 --> 00:03:46 - Amaia Lujambio

However, in this study, patients with mutation in beta-catenin that also had an inflamed phenotype were responders to immunotherapy, suggesting that even if beta-catenin mutation has been shown to be associated with a non-inflamed phenotype, it is also possible to have beta-catenin mutation and an inflamed phenotype and in that case, patients are more likely to respond to immunotherapies. This also indicates that the inflamed phenotype rather than beta-catenin mutational status may be more important to predict response to immunotherapies.

03:47 --> 04:30 - Amaia Lujambio

They also studied whether there was any association between response to immunotherapy and known HCC-related signatures and they found an association between Hoshida's S1 and S2 and response to immunotherapy. Finally, they came up with their own signature which includes genes from the IFNG pathway and antigen presentation and contains 11 genes. And these signature could be used to potentially identify those patients that are more likely to respond to immunotherapy. Interestingly, non-responders presented high levels of Tregs in the tumor microenvironment, suggesting that this cell type may be involved in the resistance mechanism.

04:32 --> 04:51 - Stephanie Rössler

This is really interesting! What I also found fascinating is that in second and third-line their signature was not related to response to anti-PD-1, suggesting that prior tyrosine-kinase-inhibitor treatments alter the immune landscape of HCC tumors in a detrimental way.

04:51 --> 05:06 - Amaia Lujambio

Yes, that's true! That also highlights the importance of having samples prior to starting immunotherapy rather than archival samples as the immune environment may have changed by TKI therapies.

05:08 --> 05:32 - Stephanie Rössler

Yes. So the second talk of the session was by Dr. Daniela Sia from Mount Sinai on "A novel microenvironment-based classification of intrahepatic cholangiocarcinoma". Cholangiocarcinoma is lagging a little bit behind the hepatocellular carcinoma that we heard from the first talk.

05:32 --> 06:02 - Stephanie Rössler

Second Park also focused on the immune microenvironment of intrahepatic cholangiocarcinoma, iCCA. This is a highly relevant study as the treatment options for patients with intrahepatic cholangiocarcinoma are still very limited. Even though targeted therapies against FGFR have received FDA approval the survival benefit remains modest and the results for the checkpoint inhibitor pembrolizumab have been disappointing in cholangiocarcinoma.

06:02 --> 06:28 - Stephanie Rössler

This is because we still do not understand how the oncogenic pathways shape the immune contexture and Therefore, it is crucial to better understand the genotype-immunophenotype relationship to design novel combination therapies.

06:28 --> 07:22 - Stephanie Rössler

To this end, they performed a virtual microdissection of a large human intrahepatic cholangiocarcinoma cohort and generated a novel classifier they named STIM classification for Stroma Tumor and Immune Microenvironment. In total, 4 different sets of gene expression data of human intrahepatic cholangiocarcinoma were used. In silico, data analysis using deconvolution of key elements of the tumor ad tumor microenvironment were extracted. 2 groups of inflamed iCCA and 3 groups non-inflamed iCCA were identified.

07:22 --> 08:00 - Stephanie Rössler

The inflamed iCCA cases had the highest numbers of CD8-T-cells and were subgrouped into the “Immune classical” subgroup which had the highest overall immune cell infiltration and into the “Inflammatory stroma” subgroup which was characterized by high desmoplastic reaction and immune cell infiltration in the stroma. Interestingly, the “Immune classical” subgroup had very few overall mutations, whereas the “Inflammatory stroma” subgroup was enriched for KRAS mutations and exhibited PI3K-AKT-MTOR pathway signaling.

08:01 --> 08:28 - Stephanie Rössler

On the other hand, the 3 non-inflamed iCCA subgroups included the largest group of iCCA which had stem cell features and therefore was termed the “Hepatic stem-like” subgroup. This subgroup had high infiltration of M2 macrophages. The “Hepatic stem-like” subgroup had the highest number of BAP1 and IDH mutations and at the gene expression level they showed NOTCH signaling activation.

08:29 --> 09:00 - Stephanie Rössler

A small group of patients had highly proliferative tumors and very low to no inflammation and this was called the “Tumor classical” subtype, whereas the last subgroup had the lowest immune cell infiltration and was therefore named: “desert-like”. The “Tumor classical” and the “desert-like” subtype both exhibited co-occurrence of KRAS and TP53 mutation and the Tumor classical” subgroup was characterized by MYC target gene expression .

09:01 --> 09:24 - Amaia Lujambio

Stephanie, the findings in patient cohorts are so important. In addition, it is important to develop relevant mouse models to test novel treatment strategies. I completely agree with you. So to this end, CIA and colleagues performed cross species analysis to identify which mouse models recapitulate the human subtypes.

09:25 --> 09:55 - Stephanie Rössler

For 3 different orthotopic mouse models they could identify the corresponding human subgroup. These mouse models were:

- KRAS overexpression together with p19 deletion,
- Notch ICD and AKT overexpression,

- YAP and AKT overexpression

09:56 --> 10:16 - Stephanie Rössler

The KRAS/p19 mouse model represented the “Inflammatory stroma”-subtype, whereas, the NICD/AKT and the YAP/AKT mouse models represented the “Hepatic stem-like” subtype of human intrahepatic cholangiocarcinoma.

10:17 --> 10:43 - Amaia Lujambio

Yeah. So it seems like there are some cholangiocarcinoma subgroups for which relevant mouse models are still missing, so the available and future mouse models will have to be characterized in regard to the patient subgroup. So their findings of mouse models representing the “Inflammatory stroma”-subtype and the “Hepatic stem-like”-subtype may be useful for testing therapeutic interventions.

10:45 --> 11:04 - Stephanie Rössler

Yes, I agree. This also highlights the need for mouse models representing the other three iCCA subgroups. It will be interesting to see in the future which subgroups can be truly represented by existing mouse models.

11:05 --> 11:29 - Amaia Lujambio

The third talk of the session was by Dr. Daniel Ho from Hong-Kong on Single-cell RNA-sequencing unravels the immunosuppressive landscape and tumor heterogeneity of HBV-associated hepatocellular carcinoma. This was a very long title! As you know, hepatitis B infection is a major risk factor for HCC development and has high prevalence in Asian countries.

11:37 --> 12:12 - Amaia Lujambio

In this study, single-cell RNAseq was performed in 8 HBV-associated HCC samples, accounting for more than 18,000 cells. One of the most interesting findings was that there was an inverse correlation between tumor-associated macrophages and tumor-infiltrating CD8 T cells. In addition, tumor-associated macrophages expressed immunosuppressive markers, a result that was validated in additional HCC patient cohorts.

12:13 --> 13:10 - Amaia Lujambio

In the case of the CD8 T cells, they were expressing exhaustion markers such as PD1 and TIGIT. And finally, they did some functional experiments where they show that the immune checkpoint landscape was modified. Then, the immune checkpoint landscape was studied and the TIGIT-NECTIN2 axis was identified as the most prominent one. TIGIT was expressed in T cells while NECTIN2 was expressed in antigen-presenting cells. To study the role of NECTIN2 in anti-tumor immunity, murine experiments were performed by using Hepa1-6 HCC cell line in vitro and in vivo. In proof-of-principle experiments, NECTIN2-KO in tumor cells was able to improve proliferation of T cells and T cell infiltration.

13:12 --> 13:59 - Stephanie Rössler

It is interesting that they inhibit NECTIN2 in tumor cells. This data suggests that a broad NECTIN2 inhibitor could have additive effects by inhibiting NECTIN2 in antigen-presenting cells and tumor cells. So the last talk of the session was by Dr. Bernd Heinrich from the NCI on “The tumor microenvironment shapes innate lymphoid cells in patients with hepatocellular carcinoma”. This

study focused on HCC to study cytokine gradients and their influence on immune cells. The authors very elegantly combined expression profiling of the triplet sample sets of non-tumor, tumor margin and tumor tissue and identified cytokines which form gradients between the non-tumor, tumor margin and invasive HCC.

14:26 --> 15:34 - Stephanie Rössler

In addition, they used human tissue cultures to measure cytokines which are released into the media by non-tumor or by tumor tissues. Cytokines that were high in the tumor center were also associated with poor outcome in the TCGA cohort, whereas cytokines with low expression in the tumor compared to non-tumor were associated with better outcome. Very interestingly, they found that Innate lymphoid cells (ILC) are the innate counterparts of T cells and are strongly influenced by these cytokines. Therefore, they used FACS analysis to identify the ILC groups that differ between non-tumor and tumor tissues. Interestingly, ILC2 cell increased from non-tumor via the tumor margin to the invasive tumor. In contrast ILC3 cells decreased from non-tumor to tumor core.

15:40 --> 13:03 - Stephanie Rössler

The cytokines produced by the tumor core were collected in fresh tissue cultures and added to mononuclear cells of non-tumor tissue. This led to an increase of ILC2 and a decrease of ILC3 similarly to the observation in tumor tissue. Lastly, single cell RNA sequencing revealed that ILC clusters differ between non-tumor and tumor tissue of HCC patients. Trajectory analysis of the single cell data further revealed that ILCs undergo plasticity from NK-like cells towards ILC2 specifically in the tumor tissue. Patients with high ILC2/ILC1 ratio undergo a stronger ILC2 directed plasticity and have a better progression free survival. This work very nicely highlights that innate lymphoid cells contribute to the anti-tumor response. NK like cells transition via ILC3 in the non-tumor tissue towards ILC2 cells in the tumor tissue which is orchestrated by key cytokines and high numbers of ILC2 cells in the tumor tissue are associated with better outcome.

17:16 --> 17:57 - Amaia Lujambio

Yeah, I I was very impressed by the use of fresh tissue and in vitro culture of immune cells. Also, this work emphasizes that detailed analysis of immune cell plasticity may help better understand and the tumor immunity. Well, thanks Stephanie. Thank you so much for helping me summarize General Session one on molecular pathogenesis from ILCA 2021. I think this four works demonstrate that we are in the right direction to better understand hepatocellular carcinoma and cholangiocarcinoma. And I I'm really looking forward to talking to you at ILCA 2022 and discussing more science with you.

17:58 --> 18:14 - Stephanie Rössler

Thank you Amaia. It was also my pleasure working together with you and it was really fun to be at ILCA 2021 and I'm really looking forward to this year ilca. And I'm hoping to see many of the colleagues.

18:17 --> 18:17 - Amaia Lujambio

Bye.

18:18 --> 18:19- Stephanie Rössler

Bye bye.