Method: Using a fluorescent in situ hybridization assay, we retrospectively evaluated the frequencies of ROS1, ALK, MET and HER2 rearrangements and or amplifications in a series of 138 BTC (62 intrahepatic cholangiocarcinomas (CC), 29 hilar CC, 15 common bile duct CC, 32 gallbladder adenocarcinomas). ROS1, ALK, MET and HER2 immunostaining was performed together.

Results: We detected HER2 amplifications in 2% (1/62) of intrahepatic CC and in 16% (5/32) of gallbladder adenocarcinomas. Anti-HER2 immunostaining was strongly positive in these 6 cases. We detected 1 MET amplification in the whole series. This case was an intrahepatic CC and was associated with a strong anti-MET immunostaining. We did not detect any other targetable molecular alterations using our panel of probes against ROS1, ALK, MET, HER2. Particularly, hilar and common bile duct CC showed no targetable molecular alteration. We observed several chromosomic alterations suggestive of chromosomal instability in 12% of BTC. Interestingly, 5 cases (all intrahepatic CC) harbored a centromeric alpha-sequences amplification.

Conclusion: HER2 amplifications are recurrent molecular alterations in BTC and seem to be more frequent in gallbladder adenocarcinomas. Immunostaining seems to be performant to detect these cases in routine practice. MET amplifications seem to be rare events. Nevertheless, due to a low rate of targetable molecular events detected in this series, we could not perform any survival analysis and this would require a larger series.

OFP-02-011
Evaluation of necroptosis related genes RIPK1, RIPK3 and MLKL-p immunogenicity in hepatocellular carcinoma

L. Nicolè[*], T. Sanavia, R. Cappellesso, V. Maffeis, V. Guzzardo, C. M. Radu, M. Guido, G. Zanus, A. Fassina
[*]University of Padova, Medicine, Italy

Background & Objective: Necroptosis is a form of programmed necrosis. When necroptosis occurring in cancer, dispersed tumoural elements may contribute to boost the immune response. Receptor-interacting protein kinases 1 and 3 (RIPK1 and RIPK3) and the mixed-lineage kinase domain-like protein (MLKL) are the main elements composing the subcellular pathway that realizes necroptosis. Aim of this study was to assess expression of RIPK1, RIPK3 and phosphorylated MLKL in a cohort of HCC patients and their correlation with infiltrating CD8+ T-cells and clinical follow-up data.

Method: RIPK1, RIPK3 and MLKL-p expression was assessed with immunohistochemistry (IHC) in 83 FFPE samples of resected HCC patients. Expression was evaluated on a 4-tired scale. Tumoural and peritumoural infiltrating CD8+ T cells were automatically assessed on digitized sections. Co-localization of necroptotic factors was verified by multiplex imaging. Wilcoxon Rank Sum test and survival analysis were applied to: 1) compare the correlation between RIPK1, RIPK3, MLKL-p and their combination with T cell-infiltration; 2) evaluate the prognostic impact of these necroptotic kinases in HCC. Results were compared with those obtained from computational analysis of RNA-seq data in 373 HCC patients from TCGA.

Results: RIPK1, RIPK3 and MLKL-p expression are significantly associated with tumoural but not peritumoural CD8+ T-cells infiltration (p-values < 6e-05 and > 0.4, respectively). By combining the IHC scores of the three kinases, the strength of the association with tumour infiltrating CD8+ T-cell increases (p-value 2.6e-09). Results are confirmed by TCGA RNAseq data.

Conclusion: Necroptosis occurs in a subsets of HCC patients and it is correlated to the entity of infiltrating CD8+ T-cells.

OFP-02-012
Immunohistochemical staining patterns of the PDAC stroma and their prognostic implications

T. Oguzsoy[*], P. Bagci, G. Akbas
[*]Marmara University, Pathology Dept., Istanbul, Turkey

Background & Objective: Our aim is to evaluate the relationship between stromal markers (Galectin-1, SMA, Collagen Type IV) and histopathological parameters of pancreatic ductal adenocarcinoma (PDAC), to investigate the role of these parameters in predicting the prognosis, and to show their relationship with response to therapy, if any.

Method: 76 consecutive resections diagnosed as PDAC, were immunostained with SMA, Collagen Type IV and Galectin-1 antibodies. Statistical analysis was performed over the semiquantitative results of immunhistochemistry (IHC).

Results: Mean survival was 17.9 months (1,6-75 months). M/F = 48/28. Male gender, high grade, and surgical margin positivity were independent poor prognostic factors. There was a significant correlation between high SMA expression, and presence of angioinvasion (p= 0.006). High Galectin-1 immunreactivity had an effect over survival independent of the N stage (p= 0.035). Although not statistically significant, Collagen Type IV high-reactive cases were found to have better prognosis (HR= 0.595), independent of the pathological stage.

Conclusion: This is the first microscopy-based study to show relationship between Collagen Type IV and PDAC. Collagen Type IV can also be included in the study objectives during antiangiogenic and antistromal treatments are being developed. SMA IHC might be helpful in determining the risk of angioinvasion and capacity of systemic spread. Unlike the literature, we showed high stromal Galectin-1 expression is a good prognostic factor independent of the pathological stage. Our findings suggest, the stroma is trying to limit the spread of the tumour.

Sunday, 9 September 2018, 17:15 - 19:15, Barria
OFP-03 | Dermatopathology

OFP-03-001
25 kgy radio-sterilised human skin graft shows effective skin regeneration in nude mice

J. Tomaz De Miranda[*], F. De Andrade Bringel, V. Protocevich, A. P. Pereira Velosa, V. Capelozzi, M. B. Mathor, W. R. Teodoro
[*]Faculdade de Medicina / USP, Ciências Medicas / FMUSP, São Paulo, Brazil

Background & Objective: There has been a growing interest in radio-sterilized skin grafting, especially in extensive and deep burns. Our purpose was to evaluate the histoarchitecture of the human skin graft irradiated during the tissue repair process in NUDE mice.

Method: Nude mice received skin grafts irradiated with dose at 25 kGy and 50 kGy and non-irradiated, submitted to euthanasia on the 3rd, 7th and 21st days after surgery. Morphometric evaluation was performed to quantify keratinocytes, fibroblasts, defense cells and blood vessels. Expression of human type I collagen, mouse type I and III collagen, identified by immunofluorescence and histomorphometry.

Results: Histological results showed that irradiated human skin has influence on cell growth. At 25 kGy, on the 3rd there was an increase in fibroblasts (41.53 ± 8.81vs21.68 ± 4.90) and inflammatory cells respectively on 3rd and 7th (115.8 ± 16.73vs11.17 ± 6.56 and 144.1 ± 19.15vs70.17 ± 23.62) in relation to non-irradiated; on the 21st the keratinocytes increased in relation to the non-irradiated (260.9 ± 69.46vs138.0 ± 40.12) and 50 kGy (260.9 ± 69.46vs0.0). On 21st, the three groups presented incorporation of the human graft, being 25 kGy better to skin regeneration with lower inclusion of the human collagen I (6.31 ± 4.34vs43.20 ± 18.78) and greater mouse collagen III (34.60 ± 10.28vs22.48 ± 10.66) in relation to non-irradiated.