1. **No.**

   FP01801012

2. **Title**

   Impact of smoking on hepatocarcinogenesis related to life-style disease

3. **Period**

   2017-2019

4. **Summary**

We clarify molecular features of HCC with aggressive behavior, and establish a molecular scoring system that predicts tumor metastasis after curative treatment. The molecular subclasses were determined using corresponding analysis followed by hierarchical clustering analysis. We found a subtype characterized by presence of TP53 mutation, high FAL and significant hypomethylation on repetitive DNA sequences, and absence of β-catenin mutation were associated with progressive tumor characteristics, and predicted shorter recurrence-free survival in patients who underwent liver transplantation. However, we did not find any association between the presence of molecular risk factors and smoking habit of the patients.

Next, we tried to identify and characterize fetal liver type-HCCs (F-HCCs) through methylome analyses. The genes that were hyper- and hypo-methylated in fetal livers were used as markers to identify F-HCCs. We classified HCCs into adult liver-type and F-HCCs based on the methylation status on these genes. A subclass of F-HCCs is characterized by increased tumor size, presence of vascular invasion, hepatitis B, and TP53 mutation. Expressions of stem/biliary cell markers were significantly higher in F-type HCC than those in adult liver-type HCC, suggesting that HCC showing methylation pattern similar to that of fetal liver represented immature phenotype.

Finally, as HCC expressing stem cell markers were known to show a resistance to conventional therapies, we explored the immune phenotype of HCC to predict the efficacy of immune checkpoint inhibitors (ICIs) in such HCCs. Expression of PD-L1 on tumor positively correlated with stem cell markers and mutations involving the PI3K-mTOR pathway. Although CD8+ cells densely infiltrated in PD-L1-positive tumors, these tumor infiltrates expressed multiple co-inhibitory receptors, suggesting T-cell exhaustion. Conversely, PD-L1-negative HCCs were characterized by mutations in β-catenin pathway and low number of CD8+ tumor infiltrated lymphocytes, although expressions of co-inhibitory receptors were rare. These characteristics should be taken into consideration for the treatment of HCC using ICIs.