**INK4A promoter methylation from circulating cell-free DNA determined by pyrosequencing evaluation as a method for diagnosis and treatment of hepatocellular carcinoma.**

Elijah Lohman, Karam Hadidi, Gengming Huang, Peter Hu, Jianli Dong

A number of cancers are diagnosed and monitored using imaging techniques. This method is limited in its diagnostic utility by the necessity for common imaging characteristics, such as venous uptake and washout characteristics, which may not be present in early tumor differentiation, limiting the technique to later stage cancers. This is especially true in Hepatocellular Carcinoma (HCC), where in tumors can only be definitively diagnosed though imaging of a mass greater than 2 centimeters that displays distinct characteristics and biopsy (a fairly invasive diagnostic technique), leading to late stage diagnosis where few treatments are available and palliative care may be the option. Findings have shown that 60%-80% of HCC show hypermethylation of the CpG islands in the promoter region of the inhibitor of cyclin-dependent kinase 4A (INK4A) gene. This becomes relevant for possibility of a non-invasive Diagnostic technique when tumor DNA is released into the surrounding tissue and plasma of the patient, during tumor proliferation and cell cycling. This DNA can be collected as cell-free DNA (cf-DNA) from patient plasma. The purpose of this research is to define levels of promoter methylation of the INK4A gene that are relevant in the diagnosis, progression (staging) and monitoring of HCC using a non-invasive technique. Promoter methylation of the INK4A CpG islands, if used in a clinical setting, may lead to earlier diagnosis and treatment of HCC, which could mean a more favorable prognosis and survival. This study investigates methylation status of the INK4A promoter CpG islands in a prospective fashion in patients that are tested for α-feto protein for the screening and diagnosis of HCC. Sample’s cf-DNA is extracted from plasma and subjected to bisulfite conversion and methylation specific polymerase chain reaction (PCR), which is then sequenced using pyrosequencing. The process of pyrosequencing determines the percentage of cf-DNA methylation of the CpG islands, which may be diagnostically relevant. Upon completion of sample collection and methylation quantification methylation levels of all samples were statistically analyzed with the patient’s diagnosis and medical information to determine clinically relevant levels. Our findings showed that detection of INK4A promoter methylation using cf-DNA can be used to enhance diagnostic sensitivity for HCC, especially when used in combination with other biochemical and imaging techniques.