

LIST OF ABBREVIATIONS

4E-BP1	: Eukaryotic initiation factor 4E binding protein 1
Ab	: Antibody
AFP	: Alpha fetoprotein
ALT	: Alanine aminotransferase
AMPK	: Adenosine-monophosphate-activated protein kinase
α-SMA	: Alfa-smooth muscle actin
AST	: Aspartate aminotransferase
Atg	: Autophagy-related protein
ATP	: Adenosine triphosphate
BCLC	: The Barcelona Clinic Liver Cancer
BDL	: Bile duct ligation
Beclin 1	: Bcl-2-interacting myosin-like coiled-coil protein
CD	: Cluster of differentiation
Cdc	: Cyclin-dependent kinase
CHC	: Chronic hepatitis C
CLIP	: The Cancer of the Liver Italian Program
CMA	: Chaperone-mediated autophagy
CNI	: Calcineurin inhibitors
CT	: Computed tomography
CTL	: Cytotoxic T lymphocytes
DAB	: 3, 3' Diaminobenzidinetetrahydrocholride
DC	: Dendritic cell
Deptor	: The DEP-domain-containing mTOR-interacting protein
DNA	: Deoxyribonucleic acid
ECM	: Extracellular matrix
eIF4E	: Eukaryotic initiation factor 4E
ELISA	: Enzyme linked immunosorbant assay
ER	: Endoplasmic reticulum
FET	: Fisher's Exact test
FKBP	: FK506-binding protein
Foxp3	: Forkhead box P3
FRAP	: FK506 binding protein 12-rapamycin associated protein
FRB	: FKBP12-rapamycin binding
G6PDH	: Glucose-6-phosphate dehydrogenase
GCN2	: General control nonderepressible 2
GGT	: Gamma glutamyltranspeptidase

GI	: Gastrointestinal
GTPases	: Guanosine triphosphatases
GβL	: G protein b-subunit-like
Hb	: Hemoglobin
HBV	: Hepatitis B virus
HCC	: Hepatocellular carcinoma
HCMV	: Human cytomegalovirus
HCV	: Hepatitis C virus
HIF-1	: Hypoxia-inducible transcription factor-1
HIV	: Human immunodeficiency virus
HMGB1	: High mobility group 1
HRP	: Horseradish Peroxidase
HSCs	: Hepatic stellate cells
hTERT	: Human telomerase reverse transcriptase
HVR	: Hypervariable regions
IFN	: Interferon
IGF	: Insulin-like growth factor
IHH	: Immortalized human hepatocytes
IKKβ	: Inhibitor of nuclear factor κB kinase β
IL	: Interleukin
iNKT cells	: Invariant natural killer T cells
iNOS	: Inducible NO synthase
INR	: International normalized ratio
IP3	: Myo-inositol-1,4,5-triphosphate
IRGM	: The immunity-associated GTPase family M
IRS-1	: Insulin receptor substrate-1
LAMP	: Lysosome-associated membrane protein
LC3	: Microtubule-associated protein light chain 3
MELD	: Model for End Stage Liver Disease
MHC	: Major histocompatibility complex
MMP	: Matrix metalloproteinase
mRNA	: Messenger ribonucleic acids
mTOR	: Mammalian target of rapamycin
mTORC	: mTOR complex
NASH	: Non-alcoholic steatohepatitis
NFκB	: Nuclear factor κB
NK	: Natural killer
Nrf2	: Nuclear factor (erythroid-derived 2)-like factor 2

NS	: Non-structural
OD	: Optical density
PA	: Prothrombin activity
PBS	: Phosphate buffered saline
PCD	: Programmed cell death
PCR	: Polymerase chain reaction
PDGF	: Platelet-derived growth factor
PE	: Phosphatidylethanolamine
PI3K	: Phosphoinositol 3-kinase
PI3P	: Phosphatidylinositol-3-phosphate
PIP2	: Phosphatidylinositol-3,4-bisphosphate
PIP3	: Phosphatidylinositol-3,4,5-triphosphate
PKA	: Protein kinase A
p-mTOR	: Phosphorylated-mTOR
PPARγ	: Peroxisome proliferator-activated receptor- γ
PRAS40	: Proline-rich AKT substrate of 40 kDa
PTEN	: Phosphatase and tensin homolog
RAFT	: Rapamycin and FKBP12 target
RAPT 1	: Rapamycin target 1
Raptor	: Regulatory-associated protein of mTOR
Rapalogs	: Rapamycin analogs
RdRp	: RNA-dependent RNA polymerase
Rheb	: Ras homolog enriched in the brain
RpS6	: Ribosomal protein S6
Rictor	: Rapamycin- insensitive companion of mTOR
RNA	: Ribonucleic acid
ROC	: Receiver-operating characteristic
ROS	: Reactive oxygen species
rRNA	: Ribosomal ribonucleic acid
S6K1	: S6 Kinase 1
SD	: Standard Deviation
SEP	: Sirolimus effector protein
SREBP-1	: Sterol regulatory element binding protein-1
STAT	: Signal transducer and activator of transcription
STK11/LKB1	: Serine/threonine kinase 11
TCA	: Tricarboxylic acid
TCR	: T cell receptor
TGF-β	: Transforming growth factor beta

Th	: T helper
TLR	: Toll-like receptor
TNF	: Tumor necrosis factor
TOR	: Target of rapamycin
TORKinibs	: mTOR kinase inhibitors
TOS	: TOR signaling
TRAIL	: Tumor necrosis factor-related apoptosis-inducing ligand
Treg	: T regulatory cells
TSC	: Tuberous Sclerosis Complex
ULK1	: UNC51-like kinase 1
UPR	: Unfolded protein response
UTR	: Untranslated region
UVRAG	: Ultraviolet radiation resistance-associated gene
VEGF	: Vascular endothelial growth factor

LIST OF TABLES

Table	Page
I. Identification of autophagy-related proteins (Atg) genes and their functions.	22
II. Clinical and ultrasonographic data of patients with chronic hepatitis C.	42
III. Clinical and ultrasonographic data of cirrhotic patients without hepatocellular carcinoma.	44
IV. Clinical and ultrasonographic data of cirrhotic patients with hepatocellular carcinoma.	45
V. Radiological tumor characteristics in cirrhotic patients with hepatocellular carcinoma.	46
VI. Mean \pm SD and frequencies of clinical and ultrasonographic data of cirrhotic patients with and without hepatocellular carcinoma (HCC).	47
VII. Mean \pm SD and frequencies of radiological tumor characteristics of cirrhotic patients with hepatocellular carcinoma (HCC).	48
VIII. Laboratory and virological data of patients with chronic hepatitis C.	49
IX. Laboratory and virological data of cirrhotic patients without hepatocellular carcinoma.	51
X. Laboratory and virological data of cirrhotic patients with hepatocellular carcinoma.	52
XI. Laboratory data of healthy subjects.	53
XII. Statistical comparisons between patients with chronic hepatitis C (CHC), cirrhotic patients with and without hepatocellular carcinoma (HCC) and healthy subjects as regards laboratory data.	54
XIII. Child-Pugh classification and the Model for End Stage Liver Disease (MELD) score in cirrhotic patients with and without hepatocellular carcinoma (HCC) and the Barcelona Clinic Liver Cancer (BCLC) stage and the Cancer of the Liver Italian Program (CLIP) stage in patients with HCC.	55
XIV. Statistical comparisons between cirrhotic patients with and without hepatocellular carcinoma (HCC) as regards Child-Pugh classification and the Model for End Stage Liver Disease (MELD) score and the frequencies of the Barcelona Clinic Liver Cancer (BCLC) stage and the Cancer of the Liver Italian Program (CLIP) stage in patients with HCC.	56
XV. Serum mammalian target of rapamycin (mTOR) levels (ng/ml) in patients with chronic hepatitis C (CHC), cirrhotic patients with and without hepatocellular carcinoma (HCC) and healthy subjects.	57

Table	Page
XVI. Histopathological findings in liver biopsies of patients with chronic hepatitis C virus (HCV) infection.	59
XVII Histopathological findings in hepatitis C virus (HCV)-related hepatocellular carcinoma (HCC) and the surrounding non-neoplastic liver tissues	60
XVIII Frequencies of histopathological findings in liver biopsies of chronic hepatitis C virus (HCV) infection, HCV-related hepatocellular carcinoma and the surrounding non-neoplastic liver tissues.	61
XIX Immunohistochemical expression of mammalian target of rapamycin (mTOR) and autophagy-related protein 5 (Atg5) in liver biopsies of patients with chronic hepatitis C virus (HCV) infection.	65
XX Immunohistochemical expression of mammalian target of rapamycin (mTOR) and autophagy-related protein 5 (Atg5) in HCV-related hepatocellular carcinoma (HCC) and the surrounding non-neoplastic liver tissues.	66
XXI Distribution of immunostaining positivity of mammalian target of rapamycin (mTOR) and autophagy-related protein 5 (Atg5) in chronic hepatitis C virus (HCV) infection, HCV-related hepatocellular carcinoma (HCC) and surrounding non-neoplastic liver tissues.	67
XXII Statistical correlations (“r” value) between serum mammalian target of rapamycin (mTOR) levels, intrahepatic expression of mTOR and autophagy-related protein 5 (Atg5) and other parameters in patients with chronic hepatitis C virus (HCV) infection.	76
XXIII Statistical correlations (“r” value) between serum mammalian target of rapamycin (mTOR) levels, intrahepatic expression of mTOR and autophagy-related protein 5 (Atg5) and other parameters in patients with hepatitis C virus-related hepatocellular carcinoma (HCC)	77

LIST OF FIGURES

Figure		Page
1.	Estimated prevalence of HCV infection by World Health Organization region.	1
2.	Hepatitis C virus (HCV) genome and proteins coded by its RNA	2
3.	Current model of the HCV life cycle.	4
4.	Natural history of hepatitis C virus.	5
5.	The primary structure of mammalian target of rapamycin (mTOR).	10
6.	The mammalian target of rapamycin (mTOR) complexes, mTORC1 and mTORC2 and their signals from diverse extracellular inputs.	11
7.	A model of how the mTOR–Raptor interaction may regulate mTOR activity in response to nutrients.	12
8.	A model of the upstream and downstream of mTOR.	13
9.	The mTOR proteins regulate the balance between protein synthesis and protein degradation.	16
10.	The process of autophagy in mammalian cells.	21
11.	Steps of autophagy induction and autophagosome formation.	23
12.	Statistical comparisons between patients with chronic hepatitis C (CHC), cirrhotic patients with and without hepatocellular carcinoma (HCC) and healthy subjects as regards serum mammalian target of rapamycin (mTOR) levels.	58
13.	Receiver operating characteristic curve (ROC) shows that the sensitivity and specificity of serum mammalian target of rapamycin (mTOR) levels in the diagnosis of hepatocellular carcinoma (HCC) were 92.9% and 100% respectively at a cut-off value of 4.55 ng/ml [Area under the curve (AUC) = 0.970].	58
14.	A core liver biopsy from a patient with chronic hepatitis C, METAVIR A2, F2 (H&E stain x200).	62
15.	A core liver biopsy from a patient with chronic hepatitis C, METAVIR A1, F2 with marked steatosis (H&E stain x100).	62
16.	A core liver biopsy from a patient with chronic hepatitis C, METAVIR A3, F3 with marked steatosis (H&E stain x100).	63

Figure	Page
17. A high power view of the previous patient (H&E x200).	63
18. Hepatocellular carcinoma, trabecular pattern, grade 3, (H&E stain) (x200).	64
19. Hepatocellular carcinoma, solid pattern, grade 4, (H&E stain) (x400).	64
20. Comparisons between liver specimens from chronic hepatitis C virus (HCV) infection and HCV-related hepatocellular carcinoma (HCC) and the surrounding non-neoplastic liver tissues as regards mammalian target of rapamycin (mTOR) staining intensity	68
21. Comparisons between liver specimens from patients with chronic hepatitis C virus (HCV) infection and HCV-related hepatocellular carcinoma (HCC) and the surrounding non-neoplastic liver tissues as regards autophagy-related protein 5 (Atg5) staining intensity	68
22. A core liver biopsy from a patient with chronic hepatitis C, METAVIR F2 showing moderate cytoplasmic staining of mammalian target of rapamycin (mTOR) antibody (HRP/DAB x200).	69
23. A core liver biopsy from a patient with chronic hepatitis C with rosette formation showing strong cytoplasmic and nuclear stain of mammalian target of rapamycin (mTOR) antibody (HRP/DAB x400).	69
24. A core liver biopsy from a patient with HCV-related cirrhosis, METAVIR F4, showing strong diffuse cytoplasmic stain of mammalian target of rapamycin (mTOR) antibody (HRP/DAB x200).	70
25. Hepatocellular carcinoma, Grade 4, showing strong cytoplasmic staining of mammalian target of rapamycin (mTOR) antibody (HRP/DAB x200).	70
26. Hepatocellular carcinoma, Grade 3, trabecular pattern showing strong cytoplasmic and nuclear staining of mammalian target of rapamycin (mTOR) antibody (HRP/DAB x400).	71
27. Hepatocellular carcinoma, Grade 2 showing strong cytoplasmic staining of mammalian target of rapamycin (mTOR) antibody and less intense stain in the surrounding non-neoplastic tissue. Notice the difference in staining pattern from the neoplastic tissue on the right and non-neoplastic tissue on the left (HRP/DAB x100).	71

Figure	Page
28. Hepatocellular carcinoma, Grade 3, showing strong staining of mammalian target of rapamycin (mTOR) in tumor tissue (right) versus weak mTOR staining in the surrounding non neoplastic tissue (left) (HRP/DAB x200).	72
29. Hepatocellular carcinoma, Grade 2, showing weak cytoplasmic stain of mammalian target of rapamycin (mTOR) (HRP/DAB x200).	72
30. A core liver biopsy from a patient with chronic hepatitis C, METAVIR F2, showing strong cytoplasmic staining of autophagy-related protein 5 (Atg5) antibody (HRP/DAB x200).	73
31. A core liver biopsy from a patient with chronic hepatitis C, METAVIR A2 F2, with moderate steatosis showing strong cytoplasmic staining of autophagy-related protein 5 (Atg5) antibody (HRP/DAB x200).	73
32. Hepatocellular carcinoma, Grade 2, showing strong cytoplasmic staining of autophagy-related protein 5 (Atg5) antibody (HRP/DAB x400).	74
33. Hepatocellular carcinoma, Grade 2, showing weak cytoplasmic staining of autophagy-related protein 5 (Atg5) antibody (HRP/DAB x200).	74
34. Hepatocellular carcinoma showing weak cytoplasmic staining for autophagy-related protein 5 (Atg5) antibody (right) versus strong Atg5 staining in the surrounding non-neoplastic tissue (left) (HRP/DAB x400).	75
35. Statistical correlation between serum mammalian target of rapamycin (mTOR) levels and serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in patients with chronic hepatitis C virus (HCV) infection.	78
36. Statistical correlation between intrahepatic mammalian target of rapamycin (mTOR) expression and serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in patients with chronic hepatitis C virus (HCV) infection.	78
37. Statistical correlation between serum mammalian target of rapamycin (mTOR) levels and METAVIR histological activity grade and fibrosis stage in patients with chronic hepatitis C virus (HCV) infection.	79
38. Statistical correlation between intrahepatic mammalian target of rapamycin (mTOR) expression and METAVIR histological activity grade and fibrosis stage in patients with chronic hepatitis C virus (HCV) infection.	79

Figure	Page
39. Statistical correlation between serum mammalian target of rapamycin (mTOR) levels and steatosis grade in patients with chronic hepatitis C virus (HCV) infection.	80
40. Statistical correlation between intrahepatic mammalian target of rapamycin (mTOR) expression and steatosis grade in patients with chronic hepatitis C virus (HCV) infection.	80
41. Statistical correlation between intrahepatic autophagy-related protein 5 (Atg5) expression and serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in patients with chronic hepatitis C virus (HCV) infection.	81
42. Statistical correlation between intrahepatic autophagy-related protein 5 (Atg5) expression and METAVIR histological activity grade and fibrosis stage in patients with chronic hepatitis C virus (HCV) infection.	81
43. Statistical correlations between serum mammalian target of rapamycin (mTOR) levels on one hand and intrahepatic expression of mTOR and autophagy-related protein 5 (Atg5) on the other hand in patients with chronic hepatitis C virus (HCV) infection.	82
44. Statistical correlation between expression of mTOR and autophagy-related protein 5 (Atg5) in patients with chronic hepatitis C virus (HCV) infection.	82
45. Statistical correlation between serum mammalian target of rapamycin (mTOR) levels and intra-tumoral mTOR expression on one hand and serum alpha fetoprotein (AFP) levels on the other hand in patients with hepatitis C virus (HCV)-related hepatocellular carcinoma	83
46. Statistical correlation between serum mammalian target of rapamycin (mTOR) levels and intra-tumoral mTOR expression on one hand and tumor maximum diameter on the other hand in patients with hepatitis C virus (HCV)-related hepatocellular carcinoma.	83
47. Statistical correlation between serum mammalian target of rapamycin (mTOR) levels and intra-tumoral mTOR expression on one hand and the Cancer of the Liver Italian Program (CLIP) stage on the other hand in patients with hepatitis C virus (HCV)-related hepatocellular carcinoma.	84
48. Statistical correlation between both serum mammalian target of rapamycin (mTOR) levels and intra-tumoral mTOR expression on one hand and tumor histological grade on the other hand in patients with hepatitis C virus (HCV)-related hepatocellular carcinoma.	84

Figure		Page
49.	Statistical correlation between intra-tumoral autophagy-related protein 5 (Atg5) expression and serum alpha fetoprotein (AFP) levels in patients with hepatitis C virus (HCV)-related hepatocellular carcinoma.	85
50.	Statistical correlation between intra-tumoral autophagy-related protein 5 (Atg5) expression and tumor maximum diameter in patients with hepatitis C virus (HCV)-related hepatocellular carcinoma.	85
51.	Statistical correlation between intra-tumoral autophagy-related protein 5 (Atg5) expression and the Cancer of the Liver Italian Program (CLIP) stage in patients with hepatitis C virus (HCV)-related hepatocellular carcinoma.	86
52.	Statistical correlation between intra-tumoral autophagy-related protein 5 (Atg5) expression and tumor histological grade in patients with hepatitis C virus (HCV)-related hepatocellular carcinoma.	86