Detection of 14q32 Rearrangements In Patients With Multiple Myeloma, Using Simultaneous FISH Analysis Combined With Immunofluorescence, Using CD138

Thesis

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اكتشاف مُراتَبات
14q32 في مرضى الورم النقوي المتعدد باستخدام تحليل التهجين التألقي في الموضع (FISH) المتواقت مع التألق المناعي باستخدام CD138

رسالة
مقدمة توطئة للحصول على درجة الماجستير في
الباثولوجيا الإكلينيكية و الكيميائية

مقدمة من

الطبيبة ليلى محمود حافظ الناقة
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To My parents,

To my beloved, caring and

understanding husband.

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Laila Annaka

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LIST OF ABBREAVIATIONS

AL	Amyloid light chain
ASCT	Autologous stem-cell transplantation
BACs	Bacterial artificial chromosomes
bFGF	Basic fibroblast growth factor
BJP	Bence Jones protein
β_2M	β_2 microglobulin
BMSCs	Bone marrow stem cells
CCND1	Cyclin D1
CD	Cluster of differentiation
cDNA	Complementary DNA
CLL	Chronic lymphoid leukemia
c-MAF	Musculoaponeurotic fibrosarcoma oncogene
c-Myc	Cellular myelocytomatosis
CNS	Central nervous system
CR	Complete response
CRP	C-reactive protein
CT	Computered tomography
DAPI	4',6-diamidino-2-phenylindole
Dkk-1	Dickkopf-related protein-1
DNA	Deoxy ribose nucleic acid
EBMT	European Group for Blood and Marrow Transplant
EFS	Event-free survival
ERK	Extracellular signal-regulated kinase
Fas L	Fas ligand
Fc	Fragment crystalliable
FCM	Flow cytometry
FICTION	Fluorescence immunophenotyping and interphase cytogenetics as a tool for the investigation of neoplasm
FISH	Fluorescence in situ hydridization
FLC	Free light chain assay
HDT	High dose therapy
HGF	Hepatocyte growth factor
HIV	Human immunodeficiency virus
HRS	Hodgkin/Reed–Sternberg cells

HS	Highly significant
IAPs	Inhibitor of apoptosis proteins
IF	Immunofluorescence
IDB	International Data Base
IFX	Immunofixation
Ig	Immunoglobulin
IgH or IGH	Immunoglobulin Heavy Chain
IGF1	Insulin-like growth factor 1
IL-1β	Interleukin-1β
IL3	Interleukin 3
IL6	Interleukin 6
IMWG	International Myeloma Working Group
IP	Immunophenotyping
IRF	Interferon responsive factor
ISS	International Staging System
JAK2	Janus kinase 2
Kb	Kilobase
LDH	Lactate dehydrogenase
LSI	Locus specific identifier
M	Monoclonal
MCL1	Myeloid cell leukaemia sequence 1
M-FICTION	Multicolor FICTION
MGUS	Monoclonal gammopathy of undetermined significance
MIP-α	Macrophage inflammatory protein- α
MM	Multiple myeloma
MMSET	Multiple myeloma SET
MPR	Melphalan, prednisone and lenalidomide
MPT	Melphalan, prednisone and thalidomide
MPV	Melphalan, prednisone and bortezomib
MRD	Minimal residual disease
MRI	Magnetic resonance imaging
MYEOV	Myeloma over-expressed gene
NFκB	Nuclear factor κ B
NS	Non-significant
OPG	Osteoprotegerin
OS	Overall survival
P	Short arm of chromosome
PB	Peripheral blood
PBS	Phosphate buffered saline
PBSCT	Peripheral blood stem cell transplantation

PC5	R Phycoerythrin Cyanin 5.1
PCD	Plasma cell dyscrasia
PCL	Plasma cell leukemia
PCLI.....	Plasma cell labeling index
PD	Progressive disease
PI3K	Phosphatidyl -Inositol 3-kinase
Plasma cells....	PCs
PLT.....	Platelet
POEMS	Polyneuropathy, Organomegaly, Endocrinopathy/ Edema, M-protien and Skin abnormalities
PR	Partial response
Q	Long arm of chromosome
RANKL.....	Receptor activator of NFκB
Rel.....	Reticolo-endotheliosis oncogene
Rev/Dex.....	Lenalidomide- dexamethasone
RTN4R.....	Reticulon 4 receptor
Runx2	Runt-related transcription factor 2
S	Significant
SCF.....	Stem cell factor
Scr.....	Stringent complete response
SD	Stable disease
SDF-1 α	Stromal derived factor-1 α
Sig.....	Significance
SMM.....	Smoldering multiple myeloma
SPEP.....	Serum protein electrophoresis
STAT3.....	Signal transducer and activator of transcription 3
TBX1	T-box1
TGF- β.....	Transforming growth factor-β
Thal/Dex.....	Thalidomide/dexamethasone
TNF	Tumor necrosis factor
UPEP.....	Urine protein electrophoresis
VAD.....	Vincristine, doxorubicin and dexamethasone
VCAM-1.....	Vascular cell adhesion molecule-1
VDJ.....	Variable Diversity Joining regions
VEGF	Vascular endothelial growth factor
VGPR.....	Very good partial response
WHO	World health organization
YACs	Yeast artificial chromosomes

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INTRODUCTION

Multiple myeloma (MM) is an incurable, but treatable, hematological disorder characterized by accumulation of malignant plasma cells within the bone marrow (BM) **(Chen et al., 2007).** Myeloma cells can induce alterations in the marrow microenvironment, which, in turn provides survival factors (IL-6, IL-15, insulin-like growth factor-1, and hepatocyte growth factor) that contribute to the resistance of myeloma cells to many anticancer drugs **(Barlogie et al., 2006).**

The MM is characterized by a clinical pentad: (a) anemia, (b) a monoclonal protein in the serum or urine or both, (c) abnormal bone radiographs and bone pain, (d) hypercalcemia, and (e) renal insufficiency or failure. Mortality rates are consistently higher among men than women in each age group **(Dispenzieri et al., 2009).**

It has been reported that, all MM patients harbor cytogenetic abnormalities sometime during the course of the disease. Frequent chromosome abnormalities in MM include 14q32 rearrangements, 13q deletion/monosomy 13, 1q duplication, 1p, 6p, 11q and 17p deletions **(Yuregir et al., 2009).**

Chromosomal 14q32 region containing the immunoglobulin heavy chain (IgH) locus has been identified as a recurrent hotspot of translocations in myeloma, with a frequency of up to 75% **(Dispenzieri et al., 2009).** They are associated with translocations involving the IgH locus at chromosome 14q32, and one of several partner genes including

CCND1 (11q13), MMSET (4p16), CCND3 (6p21), CMAF(16q32) and MAFB (20q11) **(Smadja et al., 2003)**. The IgH translocations correlate with poor prognosis in multiple myeloma **(Terpos et al., 2006).**

Identification of the recurring translocations in routine evaluation of plasma cell myeloma, therefore, provides important information that may assist in the diagnostic and prognostic assessment of such cases **(Cook et al., 2006).** Although cytogenetics is becoming a major parameter in MM, it is hampered by hypoproliferative nature of plasma cells and partial infiltrates **(Avet-Loiseau, 2007).**

The fluorescence in-situ hybridization (FISH) is probably the best method for cytogenetic assessment in MM, but it requires the identification of the malignant cells **(Avet-Loiseau, 2007).** The FISH analysis combined with simultaneous immunofluorescence (IF) using CD138 is an attractive alternative approach for evaluation of chromosomal changes in MM **(Cook et al., 2006).**

The FISH can be performed on dewaxed tissue sections, but this technique remains potentially challenging; the scoring of individual nuclei is difficult due to cellular overlap and nuclear truncation. Moreover, fixation and embedding procedures may produce artifacts in tissue, thereby interfering with DNA hybridization. Alternatively, Buno and his coworkers clearly showed that FISH using tissue imprints, cytopreps, and BM smears is an easy, rapid, and reliable method to detect abnormalities with high sensitivity and specificity **(Buno et al., 2005)**.

Aim of the Work

- To detect rearrangements of IgH locus on chromosome 14q32, using FISH analysis combined with simultaneous IF using CD138, on archival BM slides.
- To study the frequency of these rearrangements in MM patients.
- To correlate the prognostic value of these rearrangements with other known prognostic factors.