A novel human multiple myeloma cell line with genetic abnormality of 1q21 gains only and CKS1B overexpression

Ke Yi
Nuclear Radiation Injury Protection and Treatment Department, Navy Medical Center of PLA
Hui Zhang
Nuclear Radiation Injury Protection and Treatment Department, Navy Medical Center of PLAt
Yajun Zhang
Nuclear Radiation Injury Protection and Treatment Department, Navy Medical Center of PLA
Weijun Fu
Shanghai Changzheng Hospital
Jian Hou
Shanghai Jiao Tong University Medical Library: Shanghai Jiao Tong University School of Medicine
Rong Li (lirong785@hotmail.com)
Navy Medical Center of PLA

Research

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Abstract

Background

Multiple myeloma (MM) remains an incurable hematologic malignancy mainly due to its cytogenetic abnormalities. It is important to establish permanent malignant MM cell lines as effective tools to develop more effective therapies.

Results

We established and characterized a new multiple myeloma (MM) cell line CZ2 from the pleural effusion of a 70-year-old man. Using nephelometry and flow cytometry, cells with typical plasma cell morphology but not classical plasma cell phenotype were found to be non-immunoglobulin-secretary cells. FISH analysis of cells revealed a unique characteristic that there is only gain of 1q21 region(1q21+), while no other common cytogenetic abnormalities of multiple myeloma such as deletion of 17p(17p-), deletion of 13q(13q-) and translocation of IgH. In addition, the original cell line maintains its single cytogenetic abnormality. Meanwhile, CKS1B, an adverse prognostic gene which is located in 1q21 region was highly expressed in CZ2 using western blotting. Knockdown of CKS1B could reduce cell viability in addition that cleaved PARP and cleaved caspase3 would be decreased.

Conclusions

Therefore, CZ2 provides a suitable material for cellular and molecular studies of multiple myeloma with only 1q21 abnormality. The cell line characterized by gain of 1q21 and high expression of CKS1B is an important model for studies of myeloma cell growth and drug resistance during therapy.

Backgroud:

Multiple myeloma (MM) is a kind of hematopoietic disease which is characterized by proliferation of plasma cells in bone marrow[1]. Over the past decades, protease inhibitors, immunomodulators, autologous stem cell transplantation and several effective treatment strategies have been developed to improve the prognosis of MM[2]. The median survival time have been extended to 5–7 years[3, 4]. However, MM is still an incurable disease because patients inevitably relapse after a variable time of therapy[5]. The incurability of MM is closely related to its biological characteristics. International myeloma work group (IMWG) suggest that the risk-stratification of MM patients can use molecular biological anomaly markers such as 1q21 (gain), t(4;14) and (del)17p[6]. Among these cytogenetic abnormalities, 1q21 gain (≤ 3 copy numbers) or amplification (3 copy numbers) is a relatively common type in MM patients[7]. 1q21 gain or amplification occurs in 30%-40% of MM patients with initial diagnosis, while in 70% of MM patients with recurrence[8–10]. A lot of clinical researches have proved that patients with 1q21 gain or amplification

exhibit poor prognosis[11]. What's more, various genes located in 1q21 have been involved in MM, especially CDC28 protein kinase regulatory subunit 1B (CKS1B) was over expressed in malignant tumors including MM, and thought to be a factor of poor prognosis in refractory/recurrent MM patients[12]. It is inconvenient and not in time to collect a bulk of neoplastic cells from MM patients for researches. Therefore, in order to figure out the mechanism under MM with 1q21 gain or amplification thus developing efficient treatment to cure MM patients more precisely, we need permanent malignant hematopoietic cell lines be as effective tools. In the present study, we established a novel MM cell line CZ2 which is characterized by 1q21 gain only and in hope that can be utilized in the research of multiple myeloma and developing more effective therapies.

Methods:

1. Patient

This study was performed with the approval of the Ethics Committee of Chang Zheng Hospital, and informed consent was obtained in accordance with the Declaration of Helsinki. A 70-year-old male patient was diagnosed with stage III of International Staging System (ISS), stage IIIA according to Durie–Salmon (DS) staging system multiple myeloma. Bone marrow aspiration showed that plasma cells accounted for 97.5% of the bone marrow cell. His myeloma cell secreted monoclonal protein of immunoglobulin D, kappa chain. The serum IgG was 3.39 g/L, IgA 0.26 g/L, IgM 0.18 g/L. We used pleural effusion cells at the time of cell collection for this experiment.

2. Cell culture and establishment of cell line.

Pleural effusions specimens were obtained from the patient and mononuclear cells were separated by Ficoll density sedimentation. CD138 + cells were isolated using CD138 magnetic beads and cultured in RPMI-1640 medium (Gibco, USA) supplemented with 10% FBS (Gibco, USA), penicillin (100 U/mL), and streptomycin (100 μg/mL). They were cultured in an incubator under an atmosphere with 5%CO_2 at 37°C. Culture medium was exchanged every 2–3 days to maintain optimal cell growth.

3. Morphology and cytogenetic analysis.

Cytospin preparations were routinely prepared and harshly treated (87 °C for 10 minutes, then cooling to 70 °C) before stained by Giemsa to regularly assess the cell type morphology and the presence of mitoses. Cell morphology was examined using light microscopy.

4. FISH analysis

Interphase fluorescent in situ hybridization (FISH) was performed on CZ2 by using the Fluorescent *in situ* Hybridization Kit (Cytocell, UK), according to the manufacturer's instructions. Commercial FISH probes targeting 1q21, del 13q14(RB1 gene), del 17p13(TP53 gene), and t (4;14) were used. At least 200

interphase cells with intact nuclei were evaluated for each probe. Positive nuclear staining in more than 20% of the cells was considered positive.

5. Immunophenotypic analysis by flow cytometry

For immunophenotypic characterization of CZ2 cells, the following antibodies were used: CD1a, CD19, CD20, CD21, CD25, CD3, CD28, CD33, CD38, CD45, CD56, CD117, CD138, CD4, CD13, CD27, CD5, CD8, CD9, CD10, CD24, CD15, CD34, CD44, CD89, CD95, CD98, CD96, CD105, CD184, HLA-DR; cytoplasmic κ and γ light immunoglobulin (Ig) chains.

6. Immunoglobulin secretion

To measure the secretion of free immunoglobulin light chains in serum and culture medium of CZ2, the nephelometric technique was used and performed on a Siemens BNII nephelometer (Siemens Healthcare Ltd.).

7. Virus detection

The presence of Epstein–Barr virus (EBV) was examined using EBV Polymerase Chain Reaction (PCR) Fluorescence Detection Kit (Huirui Biotechnology, Shanghai, China). PCR was carried out using an LightCycler480 II (Roche, USA) for 40 cycles.

8. Western Blotting Analysis

The whole cell protein from CZ2 and other MM cells were collected and resolved in 10% SDS-PAGE gels and then electrotransferred to polyvinylidene difluoride membranes (Millipore, USA). Rabbit polyclonal anti-CKS1B antibody (1:500, Invitrogen), rabbit polyclonal anti-PARP antibody (1:1000, Beyotime), rabbit polyclonal anti-caspase3 antibody (1:1000, Beyotime) were used as primary antibodies while horseradish peroxidase-conjugated goat anti-rabbit IgGs (1:3000, Santa Cruz) was used as secondary antibodies. After treating with the secondary antibodies, protein bands were detected by enhanced chemiluminescence.

9. Cell viability analysis

The MTT assay was used to measure the cell viability of CZ2 and other MM cells. These cells were seeded in 96-well plates and transfected with CKS1B siRNA or control siRNA. After 48 h or 72 h of treatment, 10 μl of the viability reagent MTT was added to each well. The plates were incubated in an incubator at 37℃ for 2 hours. The medium was then discarded and 200 μl DMSO was added to each well. The absorbance at 570 nm was then determined with a microplate reader (Tecan, Spark, Switzerland).

Results:

1.Establishment and biological characterization of CZ2 multiple myeloma cell line

The separated cells from the patient's pleural effusion could be cultured in RPMI 1640 supplement with 10% FBS and grew as single cells or in small clusters in suspension. Their doubling time was about 48 hours; hence they could be passaged every 2 or 3 days. The cells have been frozen in liquid nitrogen and proliferated after revived. Using FACScan, we found that in exponential growth phase, cells were 44.72% in G1 phase, 5.93% in G2 phase, and 49.36% in S phase (Fig. 1). Wright-Giemsa Staining showed that the cells exhibited typical plasma cell morphology, which had large nuclei, 2–3 nuclei/cell, and basophilic cytoplasm (Fig. 2).

2.Immunophenotypic and immunoglobulin analysis

CZ2 cells didn't express typical plasma cell phenotype CD38. In addition, the expression of CD19, CD45 and CD117 are negative, while CD43, CD28, CD40, CD58 and β2 are positive. Cells were partly positive for CD10 and c-MYC and marginally positive for CD7, CD15 and CyclinD1. The CZ2 cell were found to be non-secretory cell after several generations. Furthermore, PCR result of the cells showed to be EBV negative.

3.Cytogenetic characterization

Classic cytogenetic analysis revealed that in the 41st generation of cells, 65–67 chromosomes were found with the following aberrations: XX,+1□ +1q-□ +1p-□+2□+5□+5□+6□+7□+7□+7p-□+8p-□9p+□+9q-□+10□+10□+10q-□+11□+11□+12□+12□-13□-13□-14□+15□+16□+16□+17p-□+17p-□18q-□+19□+21□+Mar1[cp10] (Fig. 3).

As the results of FISH have been included in Revised International Staging System (RISS), FISH analysis is necessary during diagnosis and treatment of MM. Using the common probes of MM, which are 1q21 probe, TP53/CEP 17 FISH probe, D13S319(13q14.3) probe, and IGH dual color probe, we found that there is only gain of 1q21 region(1q21+), while no other common cytogenetic abnormalities of multiple myeloma such as deletion of 17p(17p-), deletion of 13q(13q-) and translocation of IGH(Fig. 4). In addition, the original cell line keeps on maintaining its single cytogenetic abnormality.

4.Molecular characterization

As the MM cell line CZ2 exhibited 1q21+, the only one kind of cytogenetic abnormality, we focused on 1q21 region of the cells. There are many MM-associated genes located in the 1q21 of chromosome, and among them, CKS1B is the most widely studied gene, which is a promising immunotherapeutic target of MM. We have found that the expression of CKS1B protein in CZ2 cells is significantly increased than it in peripheral blood mononuclear cell (PBMC) of normal people by western blotting analysis (Fig. 5). Meanwhile, compared with the other MM cell line RPMI 8226, CZ2 cells expressed higher levels of CKS1B protein (Fig. 5). To explore the role of CKS1B in MM cells, we knocked down the expression of CKS1B by siRNA technology in CZ2, ARH77, H929, SKO007 and RPMI 8226(Fig. 6A). The result of MTT assay revealed that the cell viability was significantly impaired in RPMI 8226, H929, and CZ2 after 48 h-interference of CKS1B siRNA, and in H929 and CZ2 after 72 h-interference of CKS1B siRNA, which

demonstrated that CKS1B is vital important in MM cell survival (Fig. 6B). To study the role of CKS1B in cell apoptosis, we examined the expression of cleaved-PARP and cleaved-caspase3. Our results showed that the expression of cleaved-caspase3 and cleaved-PARP significantly increased in CKS1B-knockdown CZ2, ARH77, H929, SKO007 and RPMI 8226 (n = 3) ($P < 0.05$), suggesting that overexpression of CKS1B reduced cell apoptosis (Fig. 6C).

Discussion:

We established a unique myeloma cell line CZ2 which possess genetic abnormality of 1q21 gains only from the pleural effusion of a patient with multiple myeloma. CZ2 cells were found to be non-immunoglobulin-secretary cells. Meanwhile, CKS1B, an adverse prognostic gene which is located in 1q21 region was highly expressed in CZ2 using western blotting (Fig. 5). Knockdown of CKS1B could reduce cell viability in addition that cleaved PARP and cleaved caspase3 would be increased (Fig. 6). It has been reported that several MM cell lines were eventually transformed into original lymphoid cell lines due to the infection of EB virus[13, 14]. We have confirmed that CZ2 is EBV-negative cells by PCR. Drexler *et al.* have proved that the typical MM cells highly express CD138, CD38, CD28, CD58 and negatively express CD10, CD19[13]. However, CZ2 didn't express typical CD38.

Cell line is a convenient and available model to explore the mechanism of diseases especially tumors. Many advanced understandings of MM and other malignant hematopathy are achieved in immortal hematopathic cell lines. There are more than 100 MM cell lines have been established[15]. Nonetheless, various characteristics of the cell lines have not been probed clearly due to restricted technologies at the time, especially cytogenetic and molecular characterizations. What's more, the results of cytogenetic analysis have shown that lots of MM cell lines have complicated cytogenetic abnormalities. On the contrast, the CZ2 cell line not only have detailed cytogenetic results detected by karyotyping and FISH, but also exhibited purely 1q21 gains without other common MM genetic abnormalities. Therefore, CZ2 is such an ideal model to study the path mechanism of 1q21 gains in MM.

Immunotherapy is thought to be a promising treatment of tumors including MM[16, 17]. Exploring a suitable tumor-specific antigen, which is expressed in tumors but not in other normal tissues, or tumor-associated antigen, which is overexpressed in tumors but poorly expressed in normal cells, is crucial in active immunotherapy. Notably, *CKS1B, MCL1, IL6R, BCL9, ILF2* and lots of MM-associated antigens are located in the 1q21 region of chromosome. In this study, we have found that CKS1B was overexpressed in CZ2 cell line and other MM cell lines (Fig. 5), in addition, CKS1B-knockdown decreased cell viability (Fig. 6). The role of CKS1B in MM has been explored for a long time. Bock *et al.* objected that the median PFS of the CKS1B and the control groups were 15.0 months and 33.0 months ($P = 0.002$), respectively[18]. What's more, CKS1B was thought to be an adverse prognostic factor in refractory or recurrent MM[12]. Zhan et al. and Huang et al. have found that the overexpression of CKS1B in MM could increase the degradation of cyclin-dependent kinase inhibitors, such as P27 and P21, thus CKS1B induced the growth and drug resistance of MM[19, 20].

In summary, the CZ2, a novel human MM cell line with purely 1q21 gains was established, providing a characteristic model for pathological and therapeutic research of MM. The unique chromosomal abnormalities of CZ2 makes the cell line be utilized to better understand the genetic and molecular mechanism of 1q21 gains of MM.

Abbreviations

MM	Multiple myeloma
CKS1B	CDC28 protein kinase regulatory subunit 1B
FISH	Fluorescent in situ hybridization
RB1	Retinoblastoma transcriptional corepressor 1
TP53	Tumorsuppressorprotein 53 gene
Ig	Immunoglobulin
EBV	Epstein–Barr virus
PARP	Poly (ADP-ribose) polymerase
MTT	3-(4,5-di methyl thiazol-2-yl)-2,5-di phenyl tetrazolium bromide

Declarations

Authors' contributions

Project conceptualization and experimental design: LR, HJ. Performed experiments: LR, YK, ZY, FW. Data analysis: YK, ZY, FW. Writing – original draft preparation: YK, ZY. Writing – review and editing: LR, HJ. Project administration and funding acquisition: LR, HJ.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from Dr. Li Rong, PhD on reasonable ground.

Ethics approval and consent to participate

Study procedures were reviewed and approved by the ethical committee of Chang Zheng Hospital,, Damascus. Written informed consent was obtained from the subject prior to participation.

Consent for publication

Written informed consent for publication was obtained from the patient.

Competing interests

The authors declare no conflict of interest.

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Figures

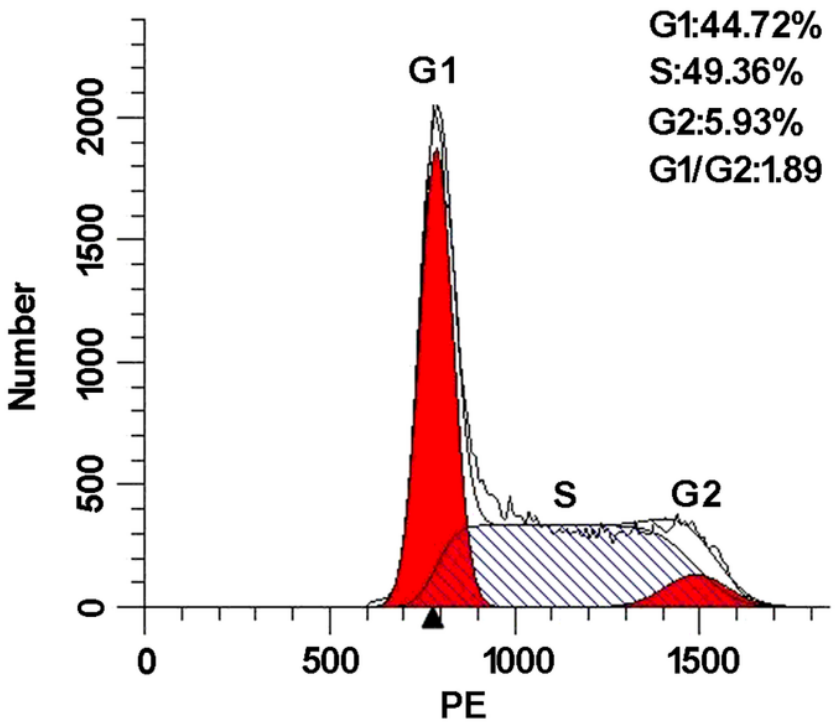

Figure 1

Cell cycle of CZ2 cell line. CZ2 cell cycle was analyzed using flow cytometry.

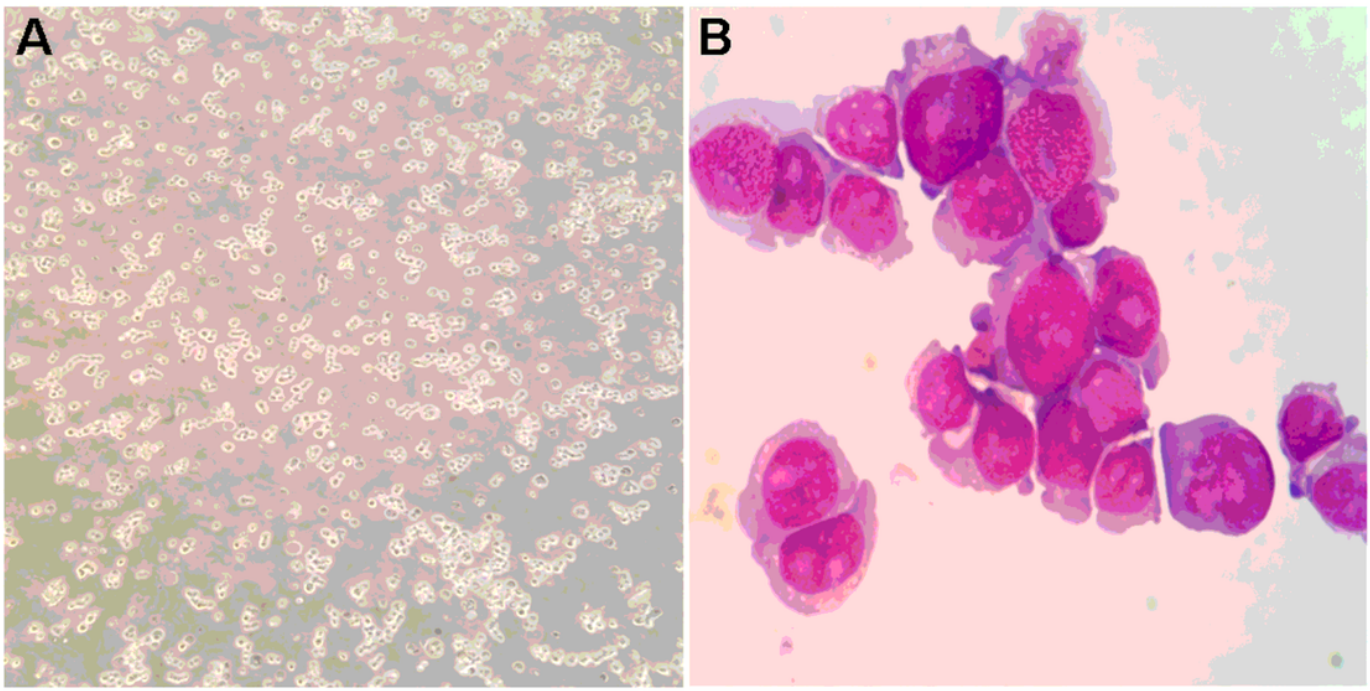

Figure 2

Cell morphology of CZ2 cell line. (A) Cultures of CZ2 cell line under the inverted microscope (100×magnification). (B) Giemsa stained cytospin slide preparations in 30th generation culture of CZ2 cell line (1000×magnification).

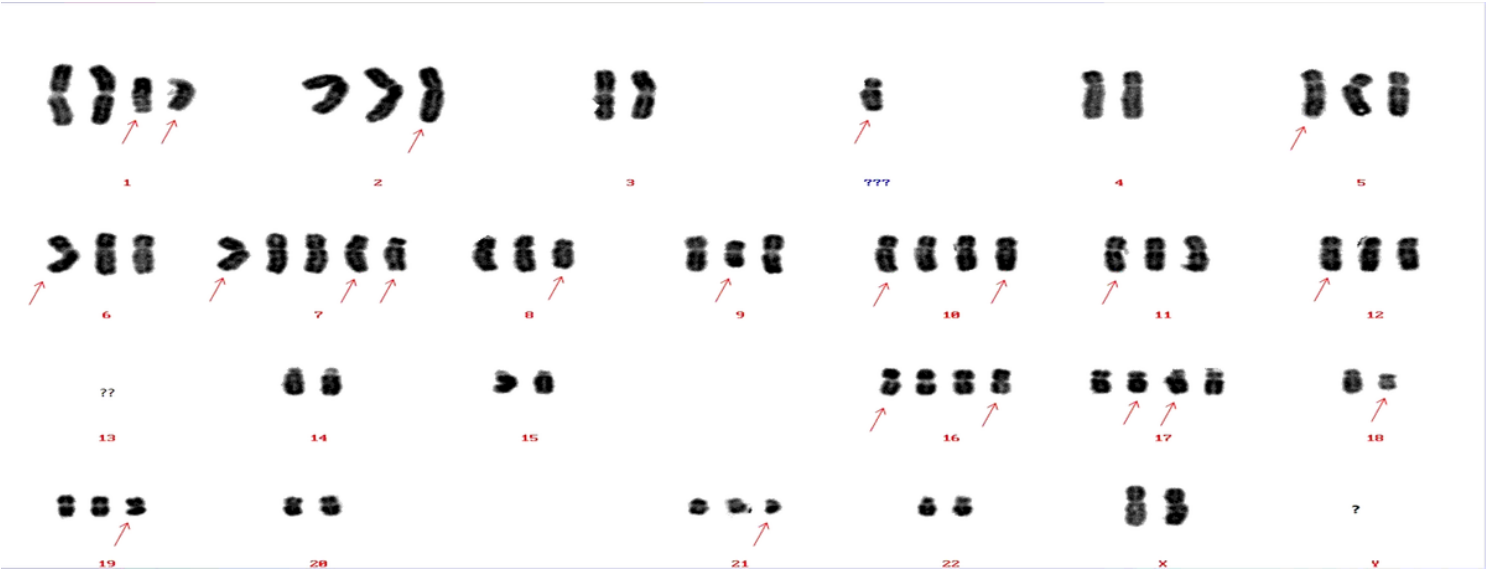

Figure 3

The karyotype of CZ2 cell line. An example of classical cytogenetic analysis.

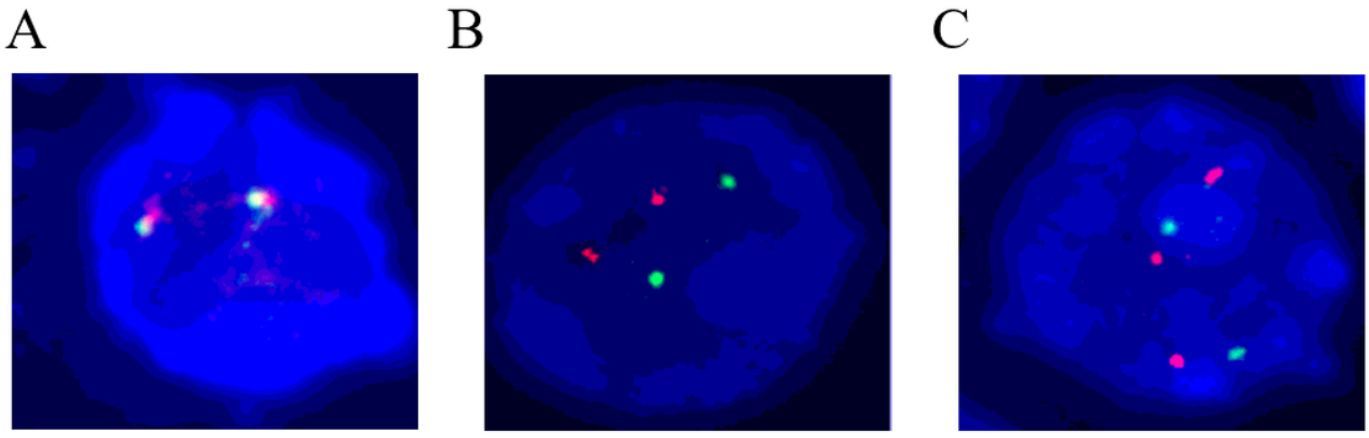

Figure 4

Result of the CZ-2 cells detected by fluorescence in situ hybridization (FISH) using the probes of the IGH, P53, D13S319, RB-1 and 1q21. (A) cells in this image show IGH were normal (2 yellow signals). (B) In this image shows normal of the P53 and D13S319(2 green and 2 red signals). (C) In this image shows normal of the RB1 but abnormal of the 1q21(2 green and 3 red) (original magnification: 10×100).

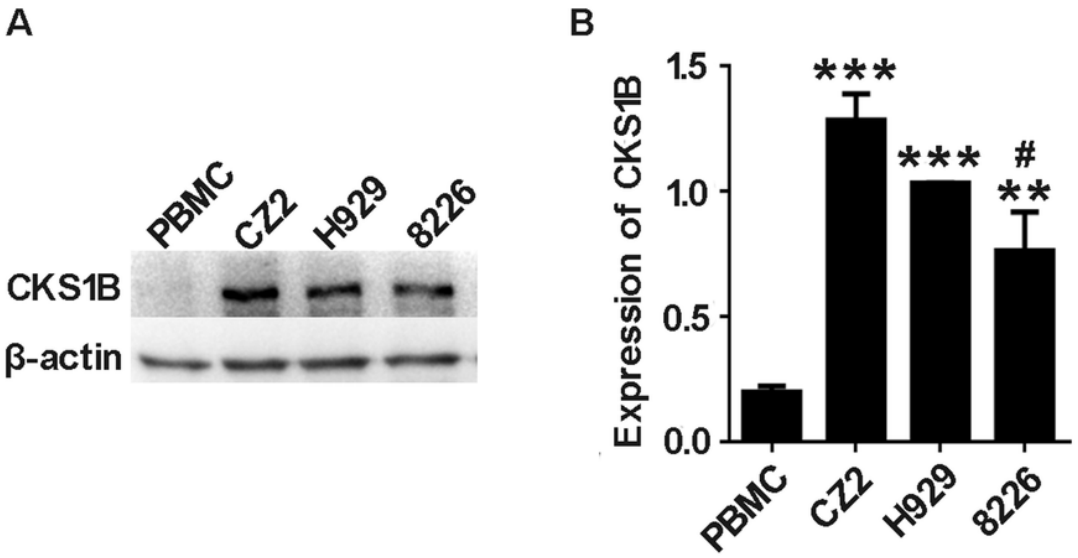

Figure 5

CKS1B is highly expressed in MM. (A) Representative immunoblot analysis of CKS1B. β-actin was used as internal standard and control for protein loading. (B) Summarized data of respective protein expression. n = 3 per group. Data are shown as the mean ± SEM. ***P < 0.001, **P < 0.01, *P < 0.05 versus the PBMC. ##P < 0.01, #P < 0.05 versus the CZ2.

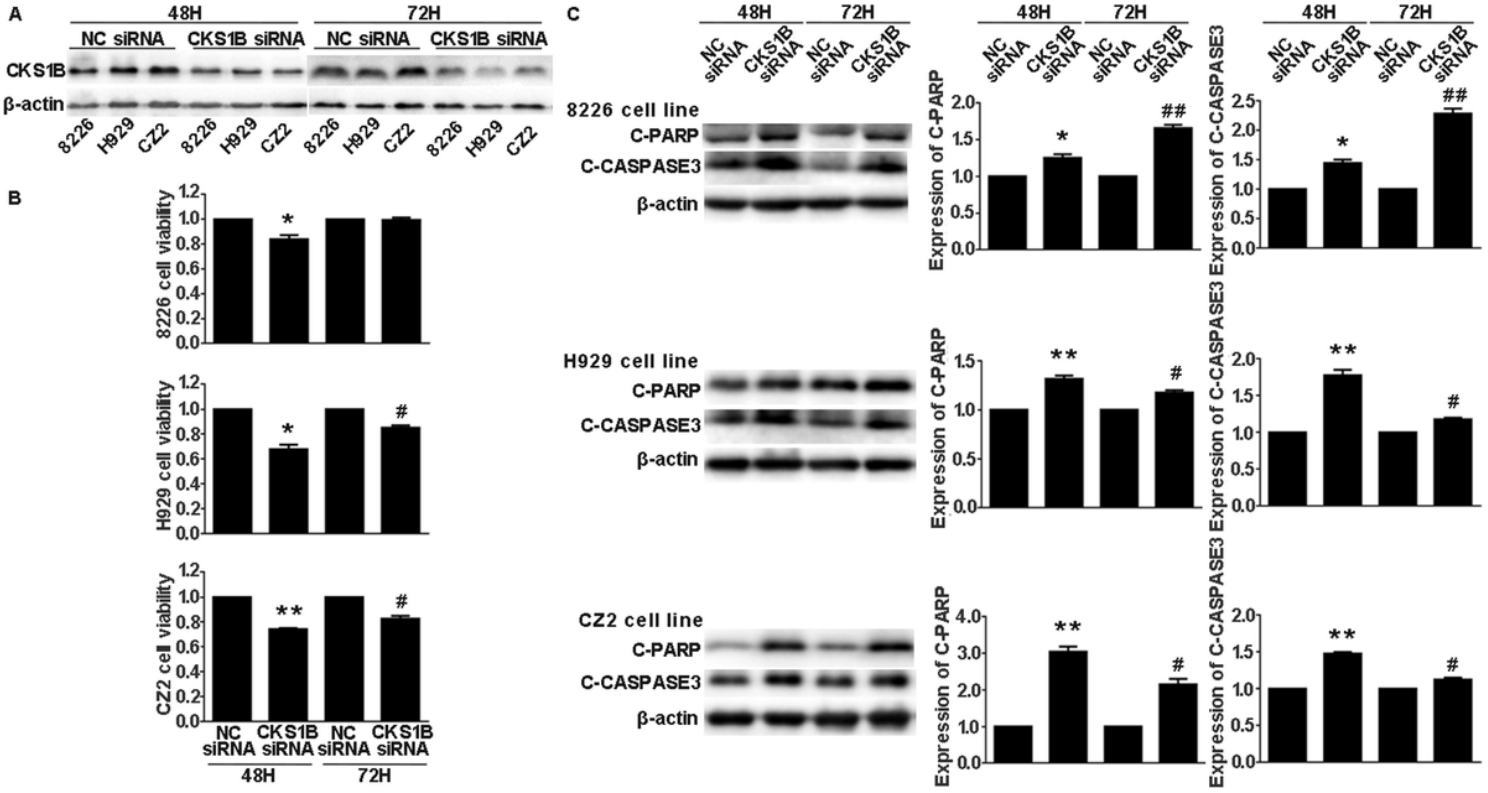

Figure 6

The MM cell viability and apoptosis after CKS1B-knock down. (A)The expression of CKS1B protein in 8226, H929 and CZ2 cells were decreased after 48h- and 72h-transfection of CKS1B siRNA compared to negative control (NC) siRNA. (B)The cell viability of 8226, H929 and CZ2 cell lines were impaired after CKS1B-knock down measured by MTT. n = 3 per group. (C) The expression of cleaved-PARP(C-PARP) and cleaved-caspase3(C-CASPASE3) in 8226, H929 and CZ2 cells were increased after 48h- and 72h-transfection of CKS1B siRNA compared to negative control (NC) siRNA. n = 3 per group. Data are shown as the mean ± SEM. **P < 0.01, *P < 0.05 versus 48h-transfected NC siRNA. ##P < 0.01, #P < 0.05 versus 72h-transfected NC siRNA.