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Invited review

Novel players in multiple myeloma pathogenesis: Role of protein kinases CK2 and GSK3

Francesco Piazza[a,b,*], Sabrina Manni[a,b], Gianpietro Semenzato[a,b]

[a] Myeloma and Lymphoma Pathobiology Laboratory, Hematologic Malignancies Unit, Venetian Institute of Molecular Medicine, Padova, Italy
[b] Hematology and Clinical Immunology Branch, Department of Medicine, University of Padova, Padova, Italy

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ABSTRACT

Multiple myeloma (MM) is an incurable plasma cell malignancy, which causes a significant morbidity due to organ damage and bone tissue destruction. In recent years, novel drugs have become available for MM therapy thanks to a more deepened knowledge of this disease's pathogenesis. The perspective of employing targeted therapies has considerably changed the expectations on the clinical outcome for patients affected by this malignancy and among the targetable molecules identified for MM therapy are several protein kinases, which have been proven to play relevant roles in supporting malignant plasma cell growth by regulating critical signaling cascades and by sustaining oncogenic mechanisms. Protein kinase CK2 (formerly known as casein kinase 2) and GSK3 (glycogen synthase kinase 3) are two multifaceted serine-threonine kinases whose task in the pathogenesis of malignant cell growth is increasingly emerging both in solid and blood tumors. In hematologic malignancies, CK2 and GSK3 have been shown to play an oncogenic function in chronic and acute leukemias as well as in MM. They have been demonstrated to act by impinging on pivotal signaling pathways that control malignant clone growth. We will herein briefly review the more recent advancements on the role of these two kinases in regulating the NF-κB, STAT3 and endoplasmic reticulum (ER) stress/unfolded protein response (UPR) signaling in MM and discuss the rationale of using small selective inhibitors as a therapeutic strategy to hamper the growth of malignant plasma cells or to improve the MM-associated bone disease.
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Contents

1. Introduction

Multiple myeloma (MM) is a blood tumor arising from terminally differentiated B lymphocytes, plasma cells, which grow mainly in the bone marrow (BM). Due to the progressive accumulation of malignant plasma cells, which produce monoclonal immunoglobulins or parts of them (light chains), end-organ damage often occurs in the bone, in the kidneys and in the bone marrow [1].

* Corresponding author at: Myeloma and Lymphoma Pathobiology Laboratory, Hematologic Malignancies Unit, Venetian Institute of Molecular Medicine, Padova, Italy; Hematology and Clinical Immunology Branch, Department of Medicine, University of Padova, Italy.. Tel.: +39 049 7923263; fax: +39 049 7923250.
E-mail address: francesco.piazza@unipd.it (F. Piazza).

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Although MM is a fatal disorder, in recent years notable progresses have been achieved in the cure of this disease, mostly due to the introduction of novel and effective drugs in the therapeutic armamentarium. For instance, the discovery that proteasome inhibitors and immunomodulatory drugs (iMIDs) caused MM cell growth arrest and their subsequent clinical employment have changed the clinical outcome of MM patients allowing to obtain a prolongation of the overall survival.

Significant efforts are being carried out in order to identify the cellular and molecular alterations underlying MM pathogenesis. As a consequence, several pre-clinical studies as well as clinical trials have started with the aim of identifying more effective therapeutic combinations able to arrest MM cell growth [2,3].

It is now well established that malignant plasma cell growth relies on cell intrinsic as well as cell extrinsic or external mechanisms. Since specific genetic lesions are associated with the deregulation of signaling pathways in MM plasma cells [4], based on their characterization it is now possible to prognostically classify MM patients [5]. Overall, a central importance in MM pathogenesis is displayed by perturbations of signaling cascades regulating the balance between cell death and life. For instance, the deregulation of Cyclin Ds, mostly D1 but also D2 and D3, is associated with a specific chromosomal translocation t(11;14) and/or specific genomic alterations. Cyclin Ds over-expression leads to increased cell cycle progression and proliferation. Also, the transcription factors c-Myc is deregulated in a substantial fraction of MM and it seems that malignant plasma cells are addicted to c-Myc overfunction. Last, the NF-κB transcription factor signaling pathway is disrupted in approximately 20% of MM cases. Different kind of mutations affecting NF-κB regulating genes altogether may converge on the over-activation of this pro-survival signaling cascade [6].

Another very important pathogenic role is played by the MM microenvironment. In the BM malignant plasma cells take crucial interactions with surrounding hematopoietic and non-hematopoietic (stromal) cells as well as with the extracellular matrix. Contacts between MM cells and the microenvironment support MM cell growth and provide a protective niche against cytotoxic agents. It is increasingly clear that the MM BM microenvironment exhibits distinct alterations, which concur to provide an inflammatory/proangiogenic *milieu* that in turn favors malignant plasma cell growth [5,7].

On the way to search for molecules involved in sustaining MM cell growth others' and our group analyzed the function of two serine-threonine protein kinases, CK2 and GSK3 in MM. These PKs are ubiquitous, regulate several cellular processes and their loss in mice is lethal.

CK2 is mostly a constitutively active kinase, even though also inducible, mainly by stress signals; GSK3, originally identified in the insulin-dependent signaling pathway as the kinase phosphorylating the enzyme glycogen synthase, is a constitutively active kinase, which is shut off in a signal-dependent fashion by upstream cascades, in particular by the PI3K/AKT axis [8,9].

CK2 and GSK3 share a common feature, *i.e.* the ability to phosphorylate several transcription factors, many of which are involved in cell proliferation, differentiation and apoptosis. For instance, both CK2 and GSK3 can phosphorylate NF-κB cascade members, promoting the activation of this pathway [10]. CK2 and GSK3 also phosphorylate c-Myc and Myb, causing an increased activity of these transcription factors [11,12]. CK2 and GSK3 have also been involved in the function of several developmentally regulated hematopoietic-specific transcription factors and chromatin modifiers [13].

Recently, a number of studies have described that both these PKs can promote MM plasma cell growth by affecting critical signaling pathways and cellular mechanisms. We will herein review the current knowledge on the pathogenic roles of CK2 and GSK3 in MM and discuss the possibility of using small selective CK2 and GSK3 inhibitors as therapeutic agents in the treatment of this disease.

2. Protein kinase CK2: a master regulator of cell survival

Protein kinase CK2 is composed by the assembly of 2 catalytic α and 2 regulatory β subunits, forming a tetramer α2β2. The alternative α′ catalytic subunit, encoded by a distinct gene, may also take part in the generation of tetramers either α′2β2 or α′αβ2. CK2 is a pleiotropic PK that has been demonstrated to be involved in a multitude of different cellular processes [14]. Nevertheless, despite its multitasking function in the cell, it has been possible to describe a prevalent role for this kinase, *i.e.* the promotion of cell survival and the protection against apoptosis [15]. Compelling experimental evidence has provided data supporting this notion, which, together with mouse models of CK2 over-expression and the observation of high CK2 levels in several tumors, allowed placing CK2 among the master regulators of cell survival. CK2 can do so by acting on a number of different mechanisms, all converging to the final output of increased cell performance against several stresses: DNA damage, oxidative stress, nutrient deprivation and so forth. Therefore, due to its central role in sustaining cell survival, it is not surprising that CK2 has been found over-expressed in many malignant solid and hematologic tumors [16]. Several studies have demonstrated that CK2 may sustain the oncogenic phenotype by impinging on tumor suppressors and oncogenes. For instance, CK2 regulates p53, PTEN, cMyc, Raf molecules, which are all important and altered in malignant tumors. Key to note, CK2 has been also shown to regulate signaling cascades, which are often dysregulated in blood malignancies. In particular, the NF-κB pathway, the PI3K/AKT and the Wnt/β-catenin signaling cascades are profoundly influenced by CK2 activity [17]. However, most of the studies up to now performed analyzed stabilized cell lines or non-malignant cells. Thus data on CK2 regulation of these specific oncogenic pathways in tissues and samples from cancer patients are still poor and only recently they have been more robustly produced in solid and hematologic tumors. In particular, works on the role of CK2 in blood tumors that have been performed in the last years have allowed identifying this kinase as a central driver of malignant blood cell pathogenesis as well as a potential therapeutic target in certain blood malignancies [17]. The first part of this review will describe the findings on the pathogenetic role of CK2 in MM.

3. Protein kinase CK2 in MM cell growth: role in the regulation of the NF-κB and STAT3 signaling pathways

MM cells depend for their growth and survival on signals arising from intrinsic and extrinsic over-activated signaling pathways. In other cell types, a number of myeloma-regulating signaling cascades have been described to be variably influenced by the activity of PK CK2. Our laboratory was thus involved in studies aimed at answering the question of whether CK2 could take part in the pathogenesis of MM. We assumed that this kinase could regulate signals originating from growth factors as well as cytokine receptors from the membrane to the nucleus of malignant plasma cells.

First, we demonstrated that a fraction of MM patients as well as stabilized MM cell lines displayed high levels of CK2, both CK2α and CK2β. Also, the kinase activity of CK2, assayed against a specific peptide, was found higher in malignant plasma cells as compared to normal bone marrow cells as well as B lymphocytes and plasma

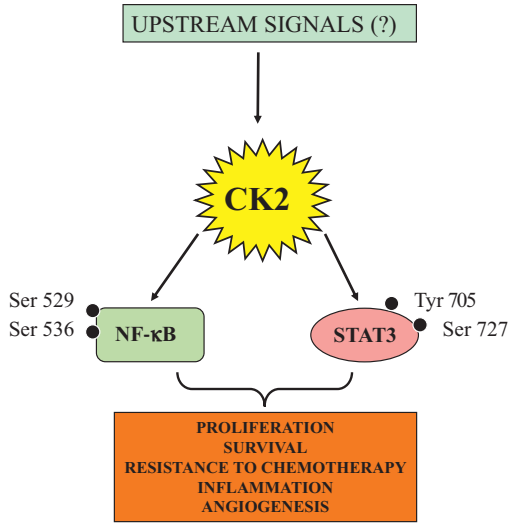

Fig. 1. CK2 action on the transcription factors NF-κB and STAT3 in MM. Protein kinase CK2 influences (through not yet clarified mechanism) the activation and transcriptional activity of NF-κB. It directly drives the phosphorylation of p65/RelA at Ser 529 and, indirectly, at Ser 536. CK2 also modulates the transmission of activation signals from the upstream kinases JAKs to STAT3, regulating the level of STAT3 phopsho Tyr 705 and Ser 727. NF-κB and STAT3 are central in tumor growth in MM by triggering the transcription of target genes involved in proliferation, survival, resistance to chemotherapy, inflammation, angiogenesis and other processes.

cells. When MM cells were challenged with CK2 small chemical inhibitors, such as tTBB (4,5,6,7-tetrabromo-1*H*-benzimidazole), its derivative K27 (2-amino-4,5,6,7-tetrabromo-1*H*-benzimidazole) and IQA ([5-oxo-5,6-dihydroindole (1,2-a) quinazolin-7-yl]-acetic acid), all of which function in a ATP-competitive manner, they displayed a significant amount of apoptotic cell death. Remarkably, normal cells (peripheral blood and bone marrow mononuclear cells) were found much less sensitive to CK2 inhibition. This was demonstrated both for MM cells lines and for MM cells isolated from the bone marrow of patients. Most importantly, the cytotoxic effect consequent to CK2 down modulation was present also in RNA interference experiments in which the CK2α catalytic subunit was silenced in MM cell lines. The general mechanism whereby CK2 inactivation caused MM plasma cell apoptosis was the triggering of both the intrinsic and the extrinsic apoptotic pathways, suggesting that this kinase lies upstream of critical survival mechanisms in malignant plasma cells. This viewpoint was strengthened by the finding that potent growth factors for MM plasma cells like interleukin 6 (IL-6) and insulin-like growth factor I, were unable to overcome the anti-proliferative effect of CK2 inhibitors. It was also shown that CK2 inhibitors were able to increase MM plasma cell sensitivity to melphalan, a central conventional chemotherapeutic agent employed in the therapy of this disease. From a mechanistic standpoint, CK2 inhibition in MM cells caused perturbations of two pivotal molecules for MM cell growth, namely the NF-κB and the STAT3 transcription factors. CK2 inhibition led to a marked reduction of IL-6-stimulated STAT3 phosphorylation, both in Tyr705 and in Ser727. Moreover, CK2 inhibitors and CK2α silencing were associated to a diminished degradation of inhibitor of NF-κB (IκB) protein at basal and TNFα-stimulated conditions and to a significant reduction of the NF-κB transcriptional activity, as determined in luciferase assays. A physical interaction between endogenous CK2α and NF-κB p105 subunit was also demonstrated in MM cells. In this first report, CK2 was clearly implicated in MM pathogenesis and for the first time it was demonstrated that CK2 regulates central pro-survival signaling pathways in malignant B cells [18]. In Fig. 1 is summarized the involvement of CK2 in NF-κB and STAT3 signaling in MM.

4. Protein kinase CK2 in MM cell growth: role in the regulation of the Hsp90 and the endoplasmic reticulum (ER) stress/unfolded protein response pathways

A subsequent paper from our group demonstrated that CK2 might impinge on the response of MM plasma cells to unfolded protein (UPR) and endoplasmic reticulum stress. This homeostatic process has recently been implicated in the development, survival and malignant growth of B-lymphocytes and plasma cells [5,19–21]. In this work, it has been shown that CK2 localizes not only in the cytoplasm but also in the ER of normal and malignant plasma cells and that the triggering of ER-stress stimulates its kinase activity. Remarkably, we described that CK2 inhibition with K27 or RNA interference of CK2α caused a stronger and earlier apoptotic effect upon exposure of MM cells to the ER stressor thapsigargin. CK2 was found to positively control the compensatory IRE1α-XBP1 arm and negatively the proapoptotic PERK-eIF2α arm of the UPR.

Based on these data and on previous work showing that CK2 controls the assembly of the chaperone Heat shock protein 90 (Hsp90) with its co-chaperone Cell division cycle 37 homolog (Cdc37) by phosphorylating Cdc37 on Ser13, we looked at the functional interaction between CK2 and Hsp90 on the regulation of the UPR. Indeed, Ser13 Cdc37 phosphorylation is instrumental for a proper recruitment of Hsp90 client proteins, in particular protein kinases and the disruption of Ser13 causes unfolding and altered maturation of several proteins [22]. In MM cells, we found that the simultaneous inhibition of CK2 and Hsp90 was synergic in causing cell death, both *in vitro* and in a mouse xenograft MM model, and produced more profound alterations of UPR pathways. In particular, CK2 plus Hsp90 inhibitors caused a intense reduction of IRE1α kinase levels in MM cells and, consequently, a lower extent of XBP-1 transcription factor mRNA splicing. Mechanistically, CK2 inhibition was associated with reduced IRE1α levels in the cell and lower levels of IRE1α/Hsp90/Cdc37 complexes [23]. The data produced in this paper suggest that CK2 might act as a protective molecule against ER stress and UPR by sustaining the homeostatic IRE1α-XBP1 dependent arm of the UPR.

Other groups have also implicated the CK2/Hsp90/Cdc37 network in MM cell survival. In the paper by Zhao et al. [24], the flavonoid compound apigenin, a CK2 inhibitor, caused MM cell apoptosis and perturbations of the Hsp90/Cdc37 axis which reflected in decreased levels of Hsp90 client proteins, such as Akt, Rip1, Raf1 and others. As a consequence, it was found that apigenin caused changes in the expression of antiapoptotic proteins Mcl1, Bcl2, BclxL, XIAP, survivin. Also, in this work, it was demonstrated a cooperative action between apigenin and Hsp90 inhibitor geldanamycin or histone deacetylase inhibitor vorinostat. Lastly, these Authors reproduced the data we previously published showing that CK2 inhibition impinged on the STAT3 and NF-κB pathways.

Key to note, other very recent reports have implicated CK2 in the ER stress response in other cell types. In prostate adenocarcinoma cells, Hessenauer et al. [25] demonstrated that CK2 inhibition with tTBB causes apoptosis and activation of the ER stress response (specifically of the transcription factor CHOP), upregulation of death receptor DR5 and sensitization to cell death by apoptosis. More recently, another group showed that CK2 modulates XBP1 activation and upregulation of Bip/Grp78 in cultured glial cells [26]. In Fig. 2 is depicted the involvement of CK2 in the ER stress response and in protein homeostasis in MM and other tumors.

Taken together, these data contribute to highlight the role of CK2 in pivotal pathways that support MM cell growth and suggest that CK2 inhibition could be a suitable strategy to be exploited in association with other agents in the therapy of this disease.

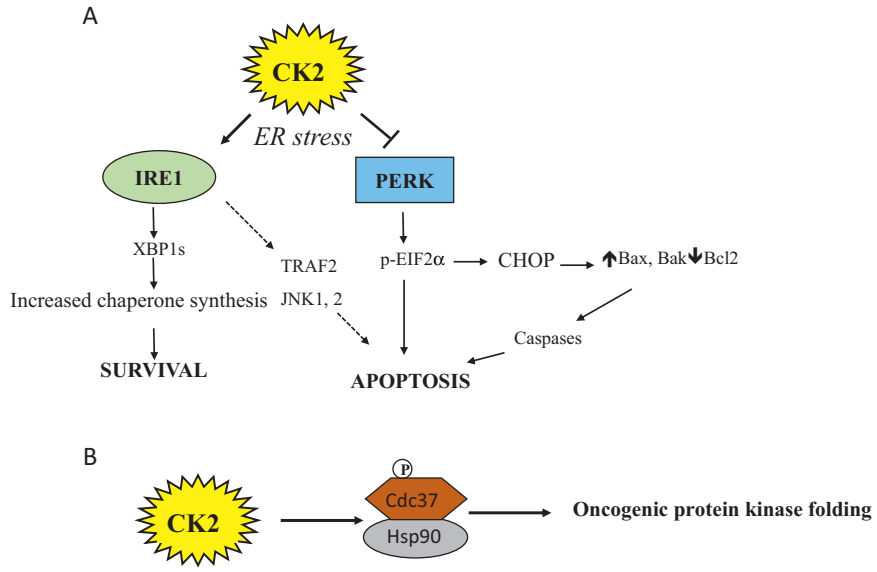

Fig. 2. Involvement of CK2 in the ER stress/UPR pathways and in the HSp90/Cdc37 chaperoning activity in MM. A. CK2 regulates the activity of the IRE1α dependent branch of the UPR, which through the activation of the transcription factor XBP1, causes an increase of the synthesis of chaperones in order to cope with the accumulation of unfolded proteins in the ER. However, IRE1α can also trigger apoptosis through the association and activation of TRAF2 and JNK1, 2. CK2 counteracts the activity of PERK/eIF2α, which if prolonged and intense, triggers apoptosis by downmodulating Bcl2 protein. B. CK2 supports the chaperonong activity of Hsp90/Cdc37 complex on cellular protein kinases. CK2 phosphorylates Cdc37 on Ser 13 and tis modification drives Cdc37 association with Hsp90 and increases the affinity of Hsp90 towards its client protiens. Among these are protein kinases which are essential for MM cell proliferation and survival.

5. GSK3: from the involvement in a "sweet" pathway to a bitter role in cancer growth

Two distinct genes, GSK3α and GSK3β, which share homology in most of the protein domains, encode GSK3. GSK3α and GSK3β might have common as well as different functions in the cell [27].

GSK3 was initially recognized as a regulative kinase of the insulin-glucose pathway. GSK3 phosphorylation of glycogen kinase and of initiation factor eIF2B causes inhibition of glycogen and protein synthesis. Upon insulin stimulation, AKT1 activation causes GSK3 phosphorylation on Ser9 (GSK3β) and Ser21 (GSK3α) and its inactivation. Consequently, glycogen and protein synthesis can start [28].

GSK3 is a peculiar kinase in that its activity is generally high in resting cells and is down modulated upon exogenous stimuli, such as growth factors, cytokines, hormones. Most of GSK3 targets are inhibited upon phosphorylation and therefore inhibition of GSK3 might result in the activation of a number of signaling cascades.

Over the years, GKS3 has been found to control important developmental and cancer-associated pathways, such as the Wnt/β-catenin, the Hedgehog and the NF-κB signaling cascades. The role of GSK3 in Wnt and Hedgehog cascades is overall of inhibiting the transmission of the signal, however, recent studies have demonstrated a positive action of GSK3 in Wnt signaling, suggesting a dual regulatory role in this cascade [29,30]. Instead, studies in GSK3β knockout mice have shown that GSK3β is essential for the activation of NF-κB upon TNFα in the hepatocytes and following studies confirmed this action also in other cell types [31].

The GSK3 substrates are many and multifold and they will not be discussed here (a number of excellent reviews are available on GSK3 function).

By virtue of its effects on Wnt, Hedgehog and growth factor-dependent signaling, the GSK3 role in cancer has been viewed as of a tumor suppressor. However, GSK3 has been described upregulated in several solid tumors and its inactivation was shown to cause cell death and reduced proliferation. It is worth mentioning that GSK3 has been shown to have an oncogenic role also in hematologic cancers, in particular multiple myeloma, acute myeloid leukemias and chronic lymphocytic leukemias [13]. In these latter tumors, the prosurvival role of GSK3 is exerted through different means. However, it is important to note that GSK3 has also been found directly or indirectly activated during apoptosis caused by many agents targeting upstream pathways. Therefore it seems that its role in malignant blood cell growth could be context and cell type-dependent. In the next paragraphs we will overview the role of GSK3 in MM growth.

6. GSK3: pro-growth role in MM

A number of studies have investigated the expression and function of GSK3 in MM cells and the data so far available must be considered early; nevertheless, they contributed important insights to prompt a more detailed analysis of this kinase.

Initial reports showed that GKS3 inhibitors were able to cause apoptosis of MM cell lines, causing dephosphorylation of forkhead transcription factors FKHRL1and FKHR and activation of the cyclin dependent kinase p27kip1 [32,33].

Another study analyzed in details GSK3-dependent regulation of the transcription factors Maf by GSK3. The Maf family is important in the pathogenesis of MM in that three genes are the target partners of the IgH locus in chromosomal translocations: cMaf in the t(16;14), MafB in t(20;14) and MafA in t(8;14) in approximately 5%, 2% and less than 1% of MM, respectively [6]. It is believed that Maf controls cell differentiation and proliferation. In this study, GSK3 was shown to be a chief kinase of MafA at residues Ser49, Thr53, Thr57 and Ser61. This phosphorylation was shown to cause a reduced half-life of MafA because of an accelerated ubiquitin-dependent proteasome degradation of the transcription factor. However, GSK3-mediated MafA phosphorylation was shown to stimulate Maf transcriptional activity by increasing the association of MafA with transcriptional coactivators. Intriguingly, GSK3-mediated Maf phosphorylation was conserved in cMaf and MafB, two Maf family members involved in MM pathogenesis. The transforming activity of Maf was demonstrated to be dependent on GSK3. Remarkably, MM cell lines treated

with GSK3 inhibitors displayed a reduction of cMaf phosphorylation [34].

Our group analyzed in detail the expression and activation status of the two GSK3 subunit, α and β in MM [35]. We found abundant expression of both the GSK3 subunits, however, in some MM patients and in all the MM cell lines analyzed GSK3β levels were lower than those of GSK3α. Interestingly, GSK3β was more abundantly phosphorylated on Ser9 than GSK3α on Ser21 in normal B cells and malignant plasma cells whereas GSK3α was fairly more abundantly phosphorylated on Tyr279 than GSK3β on Tyr217, indicating that GSK3α could be the prevailing active isoform in normal and malignant B cells. By using two different GSK3 inhibitors (SB216763 and SB415286) we also observed that GSK3 inhibition caused MM cell proliferation arrest and apoptosis. Reduction of MM cell viability was also achieved upon siRNA-directed downregulation of GSK3β, but not GSK3α. Surprisingly, instead, GSK3α downmodulation was associated with a higher sensitivity of MM cells to the cytotoxic effects of bortezomib. This latter drug was responsible of GSK3α and β partial translocation into the nucleus of MM cells preceded by Ser9 and 21 dephosphorylation. Noteworthy, GSK3 inhibition in MM cells was accompanied by β-catenin and ERK1,2 activation, two events that must be reconciled with the overall growth arrest seen. We also observed that GSK3α knockdown caused a reduction of AKT Ser473 phosphorylation and, mechanistically, we proposed a model whereby GSK3α could interfere with two bortezomib targets, the AKT and Mcl1 pathways [35].

Recently, an elegant study has provided additional important evidence on how GSK3 could promote MM cell survival. In this work, Busino et al. demonstrated, in some MM cell lines and primary samples that the non canonical NF-κB pathway is rendered overactive by the cooperation between Fbxw7α, a member of the F-box family of proteins, which function as the substrate-targeting subunits of SCF (Skp1/Cul1/F-box protein) ubiquitin ligase complexes, and GSK3. This cooperation is exerted on the physiological inhibitor of the non canonical NF-κB pathway, p100. NF-κB activation requires that p100 is removed from the nucleus by exporting mechanisms and subsequent degradation (either proteasome-dependent or through other mechanisms). p100 interaction with Fbxw7α is mediated by GSK3-dependent phosphorylation on a degron sequence encompassing Ser707. GSK3-mediated phosphorylation of Ser707 was shown to be mandatory for p100 binding to Fbxw7α and for its degradation. This process was found to be independent from NF-κB signaling, and to account for the basal turnover of p100 in the cell. NF-κB competed with Fbxw7α for the binding with p100. The importance of p100 degradation for proper non canonical NF-κB signaling was underscored by the impairment of NF-κB activation when a resistant p100 (with Ser707 mutated to Ala) was overexpressed in cells. Remarkably, p100 was found mostly cytoplasmic in human MM cells and the overexpression of a stable p100 caused MM cell growth impairment, inhibition of NF-κB gene expression and MM cell apoptosis. Most remarkable was the finding that GSK3 inhibitors caused MM cells apoptosis and that this effect was in part dependent on the raised levels of p100 in the cell [36].

We can conclude that the studies quoted above have clearly implied GSK3 as a prosurvival kinase in malignant plasma cells.

7. GSK3 in MM-associated bone disease

The role of GSK3 could be, however, even more complex. Indeed, the function of this kinase in bone developmental processes can be exploited to modulate multiple myeloma-associated bone alterations: the action of GSK3 on the Wnt/β-catenin pathway can profoundly impact on the maturation of the osteoblasts and osteogenesis. In MM this latter process is impaired by modifications induced in the BM *milieu*, and, among others, by the increased secretion of soluble Wnt inhibitors, such as Dickkopf-1 (Dkk1). Thus, bypassing the block on the Wnt cascade through the inhibition of GSK3 could represent a suitable therapeutic strategy for promoting the generation of new bone [37,38].

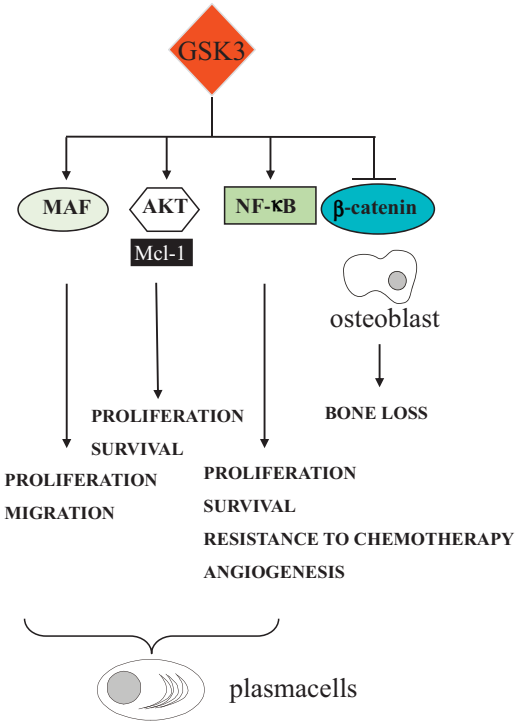

Fig. 3. Role of GSK3 in growth-promoting and bone metabolism pathways in MM. GSK3 supports MM survival and proliferation by acting in the malignnat plasma cell as well as it may favor bone loss by acting in the microenivironment. In the plasma cell, GSK3β phosphorylation of the transcription factors Maf could activates Maf-dependent transformation; GSK3α effects on Akt1, Mcl1 could influence MM cell survival and sensitivity to proteasome inhibitors; GSK3 stimulation of the non-canonical NF-κB pathway may have effects on MM cell behaviour through NF-κB-dependent stimulation of growth, inflammation, angiogenesis. In the MM microenvironment, GSK3 inhibtion of the Wnt/β-catenin pathway could impinge on the ability of the pre-osteoblast to differentiate towards osteoblast and therefore affecting MM-associated bone disease. Inibitors of GSK3 could have the dual effect of hampering MM cell growht and ameliorating bone disease.

To prove this hypothesis a very interesting paper has shown that inhibition of GSK3 can markedly reduce the myeloma-associated bone disease in a mouse model. In this work, the GSK3β inhibitor 6-bromoindirubin-3′oxime (BIO) was used. This compound selectively inhibits GSK3β and in *in vitro* experiments it was demonstrated to improve osteogenic differentiation. BIO was able to enhance the *in vivo* deposition of bone in the mouse. Moreover, BIO inhibited bone destruction and caused tumor necrosis in a xenotransplant model of myeloma bone disease. BIO also caused MM cell apoptosis *in vitro* [39,40]. A diagrammatic scheme of GSK3 in the pathogenesis of MM is shown in Fig. 3.

8. Conclusions

The pathogenesis of MM is complex and driven by alterations occurring in malignant plasma cells as well as in the MM microenvironment. Novel therapeutic approaches are designed to exert anti-myeloma effects by acting on these two levels. The search for molecular regulators of MM growth and survival is continuously progressing. Protein kinases are central in the promotion of MM cell growth [1]. The serine threonine kinases that we have herein

discussed are two novel recently identified regulators of MM biology. CK2 is a well-known survival regulator of malignant cells and only recently our group and others have implied it in the pathogenesis of hematologic malignancies. CK2 represents a very attractive therapeutic target because it sustains the activation of multiple signaling pathways, which become "addicted" to its activity (a phenomenon defined "non-oncogene addiction") [16]. Consequently, as demonstrated by compelling experimental evidence, it would be enough to taper down CK2 activity as much as to revert its over-activation to obtain malignant cell death with few alterations on normal cells. To further support this concept are the data on the *in vivo* efficacy and tolerability in human patients of the oral CK2 inhibitor CX4945 (developed by Cylene Pharmaceuticals, CA, USA), currently under scrutiny in a phase I trial in MM and other malignancies [41–43].

GSK3 is another novel player in the pathogenesis of MM and other hematologic malignancies. GSK3 also controls survival pathways, and its action could be inhibitory or stimulatory. For instance, it is well known that GSK3 inhibits the Wnt/β-catenin pathway or that this kinase counteracts the growth-promoting stimulation downstream growth factors and the PI3K/AKT cascade. On the other hand, the effects of GSK3 that strongly stimulates the NF-κB and c-Maf transcription factors have been demonstrated to account for its oncogenic role, at least in MM and other B-cell tumors, such as B-CLL [13,44]. Since GSK3 activity in the cell is produced by the two isozymes α and β, it would be of critical importance to pursue studies that, by employing genetic or molecular biology strategies, analyze individually the action of GSK3α and GSK3β, in order to dissect the distinct roles of the two isoenzymes. Clinically available GSK3 inhibitors are under development in psychiatric disorders [44], however, there is a reasonable skepticism regarding their use in cancer patients, given the tumor suppressor function of this kinase on the Wnt/β-catenin and PI3K/AKT cascades. Nevertheless, Lithium Chloride, a mood-stabilizing agent, which is widely used in the clinic and is a GSK3 inhibitor, was never demonstrated to cause an increased incidence of malignant tumors in patients, thus arguing against the possibility that inhibition of GSK3 *in vivo* could favor tumor transformation. Moreover, it is logical to envision a clinical application of GSK3 and CK2 inhibitors not as single agents but in combination therapies exploiting the effects of other synergizing drugs.

In summary, the serine-threonine kinases CK2 and GSK3 are two potential therapeutic targets in the cure of MM. It is conceivable that strategies employing CK2 or GSK3 small specific inhibitors, variably associated with conventional and novel agents, could prove in the future to be of benefit for MM patients.

Conflict of interest

The Authors declare that no conflict of interest are present.

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