Blocking clusterin inhibits emergence of resistance to zoledronic acid in osteosarcoma

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Despite recent improvements in therapeutic management of osteosarcoma (OS), new strategies are still needed to improve patient survival and to avoid emergence of treatment resistance. Zoledronic acid (ZOL) represents a promising adjuvant molecule to chemotherapy by directly affecting tumor cell proliferation. However, ZOL triggers increase of stress proteins, including Hsp27 and clusterin (CLU), which could promote tumor cell survival and resistance to ZOL treatment. We defined how CLU regulates HSF1 activity and subsequently MDR1 expression in the emergence of treatment resistance. We also found that CLU modulates the mevalonate pathway by regulating farnesyl disphosphate synthase (FDPs) expression in resistant OS cancer cells. We found that targeting CLU using antisense drug, OGX-011, suppressed treatment-induced CLU induction. Finally, combining ZOL with OGX-011 significantly reduced survival and induced apoptosis compared to either drug alone both \textit{in vitro} and \textit{in vivo}, providing a new therapeutic approach potentially translatable to patients with OS.

Keywords: zoledronic acid; clusterin; osteosarcoma; resistance; bone tumor


Osteosarcoma (OS) is an aggressive cancerous bone tumor mainly affecting children and young adults with a peak of incidence at 18 years. The usual therapeutic protocols associate a neoadjuvant poly-chemotherapy with a conservative surgery. Unfortunately, an absence of treatment response is often observed, leading to the development of metastases and the death of the patient. Survival rate depends on the treatment response reaching 70\% at 5 years for OS in the best series and only 30\% when the pulmonary metastases are detected at the diagnosis. To significantly improve survival and avoid emergence of treatment resistance in patients with OS, new therapeutic approaches targeting the molecular basis of treatment resistance in OS are needed.

We previously showed that zoledronic acid (ZOL), the third generation of nitrogen-containing bisphosphonates (N-BPs) in clinical use in treatment of bone metastasis, directly affects the proliferation and survival of OS tumor cells \cite{1,2}, making of ZOL an attractive therapy for treatment of OS by targeting both tumor cells and bone microenvironment. But, in the present study, we showed that ZOL induces a heat shock response by increasing HSF-1 activity and subsequently CLU expression, which functions as inhibitor of treatment-induced apoptosis, enhancing
emergence of treatment resistance. We next investigated the molecular mechanism that can promote emergence of resistance to ZOL in OS cancer cells by developing ZOL-resistant cell lines. We have found that this resistance progresses, at least, from selective treatment pressures that collectively enhance the apoptotic rheostat of tumor cells including up-regulation of molecular chaperones such as CLU and other HSPs. Molecular chaperones protect cells from stress-induced protein aggregation, and play key roles in transcriptional regulatory networks and cell signaling. A growing enthusiasm for therapeutic modulation of this proteostasis network highlights Hsp’s and CLU as rationale targets because of their multifunctional roles in signaling and transcriptional networks driving treatment resistance and cancer progression. In the study, we reported that CLU overexpression is induced by ZOL treatment by activating a stress response driving by the transcription factor HSF1, thus enhancing emergence of treatment resistance. CLU, a powerful chaperone, is a stress protein induced in response to a variety of insults such as oncogenic transformation and chemotherapeutic drugs. CLU expression supports tumor cells survival under lethal conditions by counteracting at various control points of the apoptotic pathway, thus enhancing the tumorigenic potential of cancer cells.

Molecular mechanisms of acquired drug resistance often involve expression of energy-dependent drug efflux pumps that detect and eject anticancer drugs from tumor cells. In the current study, we showed for the first time an increase of an ABC transporter, MDR1 in ZOL-resistant MG63 cells compared with sensitive cells. MDR1 promoter contains heat shock elements (HSE) sequences can be transcriptionally regulated by HSF1. Consequently, we found that ZOL induced HSF1 activity thus increasing MDR1 expression, while HSF1 knockdown using siRNA reduced MDR1 expression. We also demonstrated that CLU, transcriptionally regulated by HSF1, exerted a feed forward loop that in turn activated HSF1, and subsequently maintained MDR1 expression. Indeed, we confirmed that a transient overexpression of CLU significantly increased MDR1 expression. On the other hand, CLU knockdown using OGX-011 decreased HSF1 transcriptional activity, which subsequently led to decreased MDR1 expression, similar to that we observed after HSF1 knockdown. Collectively, these results highlighted a biological feedback regulation of CLU on HSF-1 activity and, indirectly on MDR1 regulation (Figure 1).

We previously described that prolonged treatment with ZOL increased FDPs, a critical enzyme involved in the mevalonate pathway that is inhibited by N-BPs expression, identifying another molecular mechanism that could explain resistance to ZOL in OS cells. In this study, we confirmed that ZOL-resistant cells exhibited higher FDPs expression than the sensitive cells and this result was correlated with
increased CLU expression. However, we demonstrated that CLU inhibition did not affect FDPs expression at transcriptional level, but decreased FDPs expression at protein level suggesting a role of the molecular chaperone CLU of FDPs pathway at protein level. The variety of described molecular mechanisms underlying N-BPs-induced resistance could be attributed to the heterogeneity of tumor cells or the methods used for inducing resistance. Thus, the main problem is to determine which molecular mechanism is involved for each patient, in order to adapt the best therapy in the era of personalized medicine.

We also demonstrated that CLU knockdown using OGX-011, a second-generation phosphorothioate antisense oligonucleotide currently in late stage clinical development, potentiated the effect of ZOL in OS cancer cells. Indeed, while ZOL increased CLU levels by enhancing HSF1 transcriptional activity both in vitro and in vivo, OGX-011 inhibited ZOL-induced CLU expression in OS cancer cells by blocking HSF1 transcriptional activity. Moreover, CLU overexpression protected, while CLU silencing enhanced, ZOL cytotoxicity in OS cancer cells. All these results suggest that CLU is a key regulator in the heat shock response regulated by HSF1. Combining ZOL with OGX-011 significantly reduced survival and induced apoptosis compared to either drug alone. Then, we used xenografts from human HOS-MNNG OS cells to translate the in vitro results to a pertinent preclinical setting. ZOL, which is currently in phase III trials in France in osteosarcoma treatment, combined with OGX-011, which is in phase III clinical trials in various cancers, cooperated to synergistically delay tumor progression and to induce apoptosis compared with each single drug, thus prolonging the overall survival. Histological analyses confirmed that the combined therapy significantly inhibited ZOL-induced CLU expression, reduced tumor cell proliferation (Ki-67) and induced apoptosis by increasing cleaved-Caspase-3 staining, corroborating with the in vitro data. In addition to synergistically enhancing anti-tumor activity, combination therapy of OGX-011 with ZOL is a good therapeutic alternative to reduce toxicity and undesirable side effects such as osteonecrosis of jaw that has been associated with ZOL in clinical trials and clinical use, by decreasing the dose of treatment. In our study, low dose of ZOL combined with OGX-011 significantly delayed tumor progression with no toxicity observed, while ZOL alone showed marginal, non-significant decrease in tumor volume.

Finally, our study demonstrated how stress induced by ZOL regulates CLU expression, and in turn, how CLU regulates HSF-1 activity, treatment resistance, and cell survival. We showed that inhibition of CLU by using OGX-011 abrogates the heat shock response induced by ZOL and synergistically cooperates with ZOL to inhibit tumor cells, thus preventing emergence of resistance. Our observations provided a preclinical scaffold for building new combined treatment targeting specific molecular mechanisms to overcome drug resistance. Our results support for the first time the development of targeted therapies employing OGX-011 combined with ZOL potentially translatable to patients.

References