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Molecular and preclinical models enhancing anti-tumour activity of zoledronic acid

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ARTICLE INFO

ABSTRACT

Zoledronic acid (ZOL) is an aminobisphosphonate able to inhibit the prenylation of intracellular proteins through the inhibition of farnesylpyrophosphate synthase. Prenylation is essential for the maintenance of the activation of components of signal transduction pathways regulating apoptosis and proliferation such as Ras and Ras-related proteins. ZOL has demonstrated a direct anti-tumour effect in vitro and in preclinical models, and its ability in preventing skeletal-related events is proven in patients with bone metastases from different origins. Clinical evidence on its direct anti-proliferative effects is emerging. We describe several strategies in order to improve the anti-tumour activity of ZOL. In detail, we illustrate new combinations between ZOL and cytotoxic drugs or other biological agents such as the farnesyltransferase inhibitor tipifarnib focusing on the sequence of administration of these drugs. Moreover, the efforts to find new molecular targets of ZOL through the use of technological platforms such as DNA microarrays are described.

1. Introduction

Bisphosphonates (BPs), synthetic analogues of the endogenous pyrophosphate molecule, inhibit osteoclast-mediated bone destruction selectively adsorbing to mineral surfaces on bone that are surrounded by osteoclasts. BPs are then released from the bone surface, where they are internalised by and disrupt the bone-resorbing action of osteoclasts.[1,2]

BPs significantly reduce the incidence of skeletal-related events (SRE), and have analgesic effects on bone pain.[3] Newer nitrogen-containing bisphosphonates, such as zoledronic acid (ZOL), have a unique mechanism of action, and are active at micromolar concentrations compared with the first-generation compounds.[4]

BPs are stable synthetic analogues of pyrophosphate. Unlike pyrophosphate which has a P–O–P central structure, BPs have a carbon atom bridging the two phosphate groups instead, giving a fundamental P–C–P backbone structure, that is essential for their biological activity.

Studies of the relationships between BPs structure and anti-resorptive potency suggested that the ability of bisphosphonates to inhibit bone resorption depends on two separate

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properties of the bisphosphonate molecule.[5] The 2 phosphonate groups, together with a hydroxyl group at the R1 position often called the 'hook', mainly influence chemical properties and pharmacokinetics imparting high affinity for bone mineral and allowing rapid and efficient targeting of bisphosphonates to bone mineral surfaces. Once localised within the bone, the structure and three-dimensional conformation of the R2 side chain (as well as the phosphonate groups in the molecule) determine the biological activity of the molecule and influence the ability of the drugs to interact with specific molecular targets.[6]

Zoledronic acid marketed by Novartis under the trade names Zometa[7] is the most potent commercially available nitrogen-containing BP to date, characterised by an imidazole side ring containing two nitrogen atoms. It is the only aminobisphosphonate indicated for the treatment of skeletal complications secondary to bone metastases derived from solid tumours including hormone-refractory prostate cancer (HRPC), breast cancer (BC), lung cancer (LC) and renal cell carcinoma (RCC).[8,9]

Patients (range 40–83 years) with bone metastases from breast and lung cancer were enrolled in order to evaluate the impact of the addition of bisphosphonates therapy to standard treatments in terms of (i) pain control, (ii) quality of life (QoL) and (iii) toxicity and to evaluate (iv) any relations between clinical activity and the occurrence of SREs.[9] The majority of patients treated with chemotherapy or hormonal therapy received ZOL 4 mg every 3–4 weeks for at least 3 cycles. No significant improvement in performance status of patients after 12 cycles of ZOL ($p = 0.1672$) was recorded. A statistically significant early and long-lasting amelioration of both pain, narcotic scores and QoL was found. An inverse correlation between bone tumour response and SREs was also found ($p = 0.019$). ZOL addition induces a clinical benefit and improves QoL of patients with bone metastases. Moreover, the occurrence of bone clinical response is related to a reduced risk of SREs.[10]

2. Molecular mechanism of action of zoledronic acid

Nitrogen-containing bisphosphonates such as ZOL interfere with the mevalonate biosynthetic pathway, and affect cellular activity and cell survival by interfering with protein prenylation and, therefore, the signalling functions of key regulatory proteins.

ZOL binds and blocks the enzyme farnesylpyrophosphate synthase (FPPS) in the HMG-CoA reductase pathway,[11] required for the synthesis of farnesyl and geranylgeranyl lipidic residues, and thereby suppresses prenylation of small GTPases that regulate the proliferation, invasive properties and proangiogenic activity of human tumour cells.[12] The addition of a lipidic residue to all the small GTP-binding proteins is essential for their correct location on the inner side of the plasma membrane and for their consequent activation by external signals. This phenomenon is known as prenylation, and is important for proper sub-cellular protein trafficking (see 'lipid anchored protein' for the principles of this phenomenon).[13]

Isoprenoids are derived from the mevalonate pathway that starts with the conversion of 3-hydroxy-3-methylglutaryl-

CoA to mevalonic acid, catalysed by the rate-limiting enzyme, 3-hydroxy-3-methylglutaryl-CoA reductase. The pathway triggered by this reaction can lead to the synthesis of a key isoprenoid molecule, the farnesylpyrophosphate (FPP) whose formation is catalysed by the farnesylpyrophosphate synthase (FPPS). FPP can either be converted by a series of reactions in cholesterol or be transferred on target cellular proteins as FPP itself (reaction catalysed by farnesyl-transferase), or is first converted into geranylgeranyl pyrophosphate and then transferred to cellular proteins by type I or type II geranylgeranyltransferase. The incorporation of lipid molecules within GTPases is important for their targeted localisation and anchorage on the inner side of the cell membrane and for consequent signal activation. Upon activation, small GTPases play important roles in cellular processes required for osteoclastic bone resorption including cytoskeleton actin structure, membrane ruffling, vesicle transport and signalling pathways that regulate apoptosis.[14]

Zoledronic acid are the more specific inhibitor of FPPS, affecting the synthesis of both farnesyl and geranylgeranyl lipid residues.[12,14]

While inhibition of protein prenylation may affect many proteins found in an osteoclast, disruption to the lipid modification of Ras, Rho, Rac proteins has been speculated to underlie the effects of bisphosphonates. These proteins can affect both osteoclastogenesis, cell survival and cytoskeletal dynamics. In particular, the cytoskeleton is vital for maintaining the 'ruffled border' that is required for contact between a resorbing osteoclast and a bone surface.[15]

Recently, Monkkonen group has hypotesised and alternative NBPs mechanism of action.[16] In detail, the FPPS inhibition leads to the upstream generation and accumulation of the ATP analogue triphosphoric acid 1-adenosin-5-yl ester 3-(3-methylbut-3-enyl) ester (ApppI) that is able to induce in vitro osteoclast apoptosis via the inhibition of mitochondrial ADP/ATP translocase.[16] Furthermore, the same group have recently demonstrated the in vivo existence of ApppI in murine peritoneal macrophages following zoledronic acid administration.[17]

3. Anti-tumour activity of zoledronic acid

Anti-tumour activity of different BPs has been demonstrated mainly in breast, prostate and myeloma cell lines. In vitro studies have shown clear anti-tumour effects of BPs, particularly zoledronic acid, as demonstrated by induction of tumour cell apoptosis and inhibitory effects on tumour cell adhesion, invasion, tumour cell viability and proliferation and angiogenesis.

There are several published reports describing the in vitro pro-apoptotic effects of BPs on osteoclasts and tumour cells.[18,19] Zoledronic acid-induced tumour cell apoptosis has been demonstrated to be associated with the release of cytochrome c and resulting activation of the caspase pathway via the inhibition of the mevalonate pathway and the consequent inhibition of prenylation of essential signalling G proteins such as Ras, Rac and Rho. In support of this, the mevalonate intermediates GGOH and FOH are able to inhibit the zoledronic acid-induced suppression of protein prenylation, activation

of the caspase pathway and apoptosis in many human cancer cell lines.

One of the primary mechanisms responsible for the direct anti-tumour activity of bisphosphonates is induction of tumour cell apoptosis. Recently we have demonstrated that ZOL induces apoptosis and growth inhibition in human epidermoid cancer cells, together with depression of Ras signalling and of Erk and Akt survival pathways.[19] These effects occurred together with poly (ADP ribose) polymerase (PARP) fragmentation and the activation of caspase-3.[20] Moreover, the latter seemed to be essential for apoptosis induced by NBPs in this experimental model. The synthesis of isoprenoids appeared largely responsible for the biological and biochemical effects of NBPs since the addition of farnesol, which restores farnesylation, to tumour cells completely antagonized apoptosis and the inhibition of Ras activity in tumour cells exposed to NBPs.[20] These data suggest that the activity of NBPs could be due to the inactivation of the FPPS activity.[20] Moreover, it was reported that ZOL induced growth inhibition on both androgen-dependent LnCaP and androgen-independent PC3 prostate cancer cell lines[21] with G1 accumulation.

Senaratne et al. showed that the effects of ZOL in human breast cancer cell lines (MDA-MB-231 and MCF-7) were associated with cytochrome c release from the mitochondria, induced by modulating expression of Bcl-2 and subsequent caspase-3 activation. These events might be precipitated by inhibition of Ras activation, which requires protein farnesylation.[22] ZOL has also been shown to cause tumour cell accumulation in the S phase of the cell cycle.[23]

Recently, it has been investigated the role of caspase-2 in the apoptosis induced by ZOL.[24] In Casp2(–/–) mouse embryonic fibroblasts, for the absence of caspase-2, Bid and Bax activation, and cytochrome c release are significantly delayed following drug treatment to indicate that caspase-2 is required for apoptosis induced by cytoskeletal disruption.[24]

Recent research indicated for the first time a ZOL anti-tumoural effect and apoptosis on primary tumours and visceral metastases. In human colon carcinoma HCT-116 cells ZOL strongly inhibited the proliferation paralleled to a G1 cell cycle accumulation and to an induction of apoptosis via a caspase dependent mechanism.[25]

However, there is little published preclinical information to support the clinical benefits of ZOL for renal cancer metastatic to bone. Pandha et al. observed its marked anti-proliferative and apoptotic effects in three renal carcinoma cancer cell lines through non-mitochondrial pathways but it was also associated with high degrees of cellular stress, as evidenced by the induction of mismatch repair protein and superoxide dismutase.[26]

Several in vitro studies have shown that BPs inhibit adhesion of tumour cells to extracellular matrix (ECM) proteins, thereby impairing the process of tumour cell invasion and metastasis.[27,28] Data reported by Wood et al. indicate that inhibition of tumour cell adhesion to ECM proteins is dependent on inhibition of protein prenylation. Therefore, inhibition of the mevalonate pathway and induction of caspase activity are important for the inhibitory effects of the bisphosphonate compound zoledronic acid. Furthermore, it has been shown that an activating Ras mutation enhanced the adhesion of a normal breast epithelial cell line to ECM

proteins, suggesting that increased Ras activation may increase the metastatic potential of breast cancer cells.[29] Thus, by inhibiting protein prenylation and Ras signalling, ZOL should reduce the ability of tumour cells to expand once they colonise bone.[28]

In addition to direct anti-tumour mechanisms, NBPs might modulate the immune system to target and eliminate cancer cells. In particular, NBPs induce significant dose-dependent expansion of γT cells both *in vitro* and *in vivo*, mainly affecting the Vγ9Vδ2 subset. T-cells expressing the Vγ9Vδ2 T-cell receptor play an important role in immune system surveillance and defense. In fact, γδT cells showed potent MHC-unrestricted lytic activity against different tumour cells in vitro, suggesting their potential utility as anti-cancer therapy.[29]

In vitro and *in vivo* studies have shown that ZOL and other NBPs have anti-angiogenic effects.[30,31] *In vitro* assays showed that ZOL could inhibit the proliferation of human umbilical vein epithelial cells induced by fetal calf serum and basic fibroblast growth factor (bFGF) in a dose-dependent manner. These findings have been confirmed *in vivo*; systemic administration of 3 μg/kg zoledronic acid to mice resulted in potent inhibition of angiogenesis induced by subcutaneous implants impregnated with bFGF.[32] The inhibitory effect of ZOL on endothelial cell adhesion and migration is mediated, at least in part, by modulation of integrins that are involved in angiogenesis.[33] More recently, Bellahcène group[34] have hypothesised that ZOL alters the recruitment to focal adhesion sites of integrins alphavbeta3 and alphavbeta5 involved in angiogenesis. In fact, ZOL generated a significant decrease in alphavbeta3 and alphavbeta5 integrin expression at HUVEC cell surface treated with zoledronate whether this mechanism of action also applies to metastatic tumour cells is under investigation.[34]

However, the role of ZOL as anti-angiogenic agent is no certain, in fact unlike previous studies that demonstrated the anti-angiogenic activity exerted by ZOL together with the inhibition of tumour cell bone invasiveness by a transient reduction of pro-angiogenic growth factors, such as VEGF, bFGF and MMP-2 circulating levels,[35] a very recent paper reports that ZOL did not appear to exert an angiogenic activity as there was no reduction of VEGF and bFGF circulating levels after zoledronic acid infusion.[36]

4. New strategies to potentiate zoledronate anti-tumour effects

It is important to consider the pharmacokinetic properties of ZOL that limit its potential anti-tumour activity *in vivo* by not allowing the achievement of anti-tumour concentrations at extra-skeletal sites. There is a rapid elimination from plasma resulting from renal excretion and rapid uptake and accumulation within bone.[37] At 4 mg intravenous dose of ZOL administrated over 15 min results in a sharp increase in its concentration, as shown by estimated distribution and elimination of the drug which results in a peak plasma concentration of ZOL (C_{max}) of approximatevely 1M. The rapid decline in plasma concentrations, falling to <1% of C_{max} by 24 h post-dose, is followed by prolonged very low drug plasma concentrations (terminal elimination half-life, t1/2γ,

approximately 7 days), typical of a BP pharmacokinetic profile.[37–39] Therefore, because of the most important limits of ZOL, its pharmacokinetic profile, further efforts are required in order to allow the clinical translation of experimental results recorded to date and to increase the anti-cancer activity of these drugs.

4.1. *Combination with other drugs*

Evidence from *in vitro* and *in vivo* models indicates that ZOL synergises with a variety of anti-cancer agents including chemotherapeutic drugs, molecular targeted agents, and other biological agents.

Based on their ability to inhibit crucial processes of protein isoprenylation ZOL and other NBPs have been combined with different biological agents. In fact, the prenyltransferases are not strictly specific, and a small G protein can be the substrate for different enzymes.

We have recently used the farnesyltransferase inhibitor (FTI) R115777 together with ZOL, and evaluated the effects of the combinatory treatment on growth inhibition and apoptosis of epidermoid cancer cells. ZOL and FTI given in combination were strongly synergistic since a CI50 (the combination index of the two drugs calculated for 50% cell survival by isobologram analysis) less than 0.5 was recorded with the dedicated software Calcusyn. Notably, low concentrations of FTI induced a strong increase of Ras expression with only a moderate reduction of Ras activity that was, on the other hand, significantly reduced by the combined treatment.[20] Moreover, ZOL/FTI combination allowed the compounds to be active in terms of tumour cell growth inhibition at *in vivo* achievable therapeutic concentrations (0.1 μM range for both drugs).[20] These data suggest that escape mechanisms for the inhibition of isoprenylation of Ras might be based on the geranylgeranylation or other prenylating processes.[38] The addition of farnesol to cells treated with the combination abolished the effects of the BPs/FTI combination on apoptosis and on the activity of the signalling molecules. These data suggest that the synergistic growth-inhibitory and pro-apoptotic effects produced by the NBP/FTI combination involve the inhibition of both Erk and Akt survival pathways acting in these cells in a Ras-dependent fashion.[20]

A synergistic interaction between R115777 and ZOL was also found on both androgen-independent PC3 and androgen-dependent LNCaP prostate cancer cell lines.[40] These effects were paralleled by disruption of Ras → Erk and Akt survival pathways, consequently decreased phosphorylation of both mitochondrial bcl-2 and bad proteins, and caspase activation. Moreover, ZOL/R115777 combination induced cooperative effects also *in vivo* on tumour growth inhibition of prostate cancer xenografts in nude mice with a significant survival increase.[41] These effects were paralleled by enhanced apoptosis and inactivation of both Erk and Akt. In conclusions, the combination between ZOL and FTI leads to enhanced anti-tumour activity in human prostate adenocarcinoma cells likely through a more efficacious inhibition of Ras-dependent survival pathways and consequent bcl-related proteins-dependent apoptosis.[42]

NBPs and chemotherapy have increasingly gained favour in the treatment of metastatic hormone resistant prostate and breast cancer. Evidence of synergistic anti-tumour effects of ZOL in combination with other agents in preclinical breast cancer studies was recently reviewed by Winter et al.[42]

Recent evidence in multiple animal models and in clinical studies has supported the biologic relevance of the anti-tumour effects of ZOL. In nude mice bearing LuCaP tumours, ZOL enhanced the cytotoxic effect of docetaxel (DTX) inhibiting significantly the growth of tumour cells in the bone. Ullen et al.[43] reported results from morphologic analyses demonstrating that the anti-tumour effects were mainly attributed to the inhibition of proliferation.

We have preliminary results about sequence-dependent synergistic effects of ZOL and docetaxel combination on growth inhibition and apoptosis of human prostate cancer cells. We have found a synergistic growth inhibition when DTX was administered 24 h before ZOL. On the basis of these results, we have designed a phase I clinical study on the combination between these two drugs metronomically administered in two different sequences in hormone-refractory advanced prostate cancer patients. The aim of this study was to perform a pharmacodynamic evaluation of the effects of the two sequential combinations through the dosage of serum angiogenic, immunologic and bone factors, and through the study of both lymphocyte sub-populations and modification of isoprenylation of intracellular proteins. Final end-point of the study was the evaluation of information for further clinical development of this combination, such as toxicity, as well as information about the mechanism of action of the combination to be translated in the preclinical setting. We have completed the first level without significant toxicities, and we have enrolled the first three patients of the second level. In the first level, we have completed the 12 cycles in only one patient, and 42 cycles were globally administered. The serum level changes of biological markers were determined before, during and after the pharmacological treatment. Interestingly, in the patient who completed the 12 cycles of therapy a significant reduction of both interleukin-8 (a pro-angiogenic factor) and -6 (a prostate cancer growth factor) was recorded. Similar results were obtained in another patient with SD. These findings were also paralleled by a significant reduction of PSA. We are now completing the evaluation of a panel of circulating angiogenic factors (interleukin-8 and -12, VEGF, PDGF), cytokines (TNF alpha, IFN gamma, GM-CSF, RANTES, interleukin-1, -2, -6 and -4) and chromogranin A. We have also collected peripheral blood mononuclear cells on which we will investigate T lymphocyte subpopulation distribution and the isoprenylation of the chaperone protein HDJ2. On the basis of the results of this study, we will design a phase III clinical study based on the 2 sequences of administration.

Moreover, in a pilot study to assess the safety and efficacy of DTX and ZOL in patients with metastatic hormone-refractory prostate cancer, the combination therapy decreased serum prostate-specific antigen levels by >50%, and resulted in a concurrent improvement in symptoms.[44]

In a phase II trial of estramustine, ZOL, and DTX in patients with metastatic androgen-independent prostate cancer patients had prostate-specific antigen decreases of >50% for a duration of 5–63 weeks and a clinically notable reduction in pain, suggesting that this combination therapy can delay dis-

ease progression in this setting.[45] Further investigations into the potential synergistic effects that docetaxel and zoledronic acid may have on cancer cells in the bone microenvironment are necessary. Finally, a new orally available mTOR inhibitor RAD001 (Everolimus) inhibits growth of prostate cancer in the bone, and the inhibitory effects are increased by combination with DTX and ZOL. Moreover, RAD001 had a significant impact on maintenance of body weight. RAD001 may hold promise for its effects on both metastatic CaP and the important syndrome of tumour cachexia.[46]

The findings of synergy of interaction between ZOL and other agents could reduce the ZOL concentrations required for anti-tumour activity, and could allow the achievement of effective *in vivo* levels.

4.2.   *New molecular targets*

New technological platforms are required to drive molecular research and to identify suitable targets of BPs action and understand cell consequences of the inhibition of the target enzymes.[47,48] Technological advances are useful for research strategy planning and technologies such as DNA microarrays, and proteomics is particularly useful in this regard. These technologies could represent a relevant opportunity to analyse the post-transcriptional modifications induced by BPs treatment, and to better understand the *in vivo* intra-tumoural molecular pathways involved in the response to these compounds. Moreover, protein microarrays might allow discovery of new BPs targets, identification of new biomarkers predictive of response, and analysis of specific molecular profiling related to the clinical response to bisphosphonate-based therapy. Identification of critical interactions within the proteome network is a potential starting point for drug development, and will aid the design of individual tailored therapies or identification of new molecular profiles, gene profiles, or both that are predictive of response to BPs-based therapy. We have preliminary results[47] about the gene modulation induced by ZOL in androgen-resistant prostate cancer PC3 cell line analysed with cDNA microarray platform. ZOL-treated cells were obtained by Affymetrix HG-U133 chips (including more than
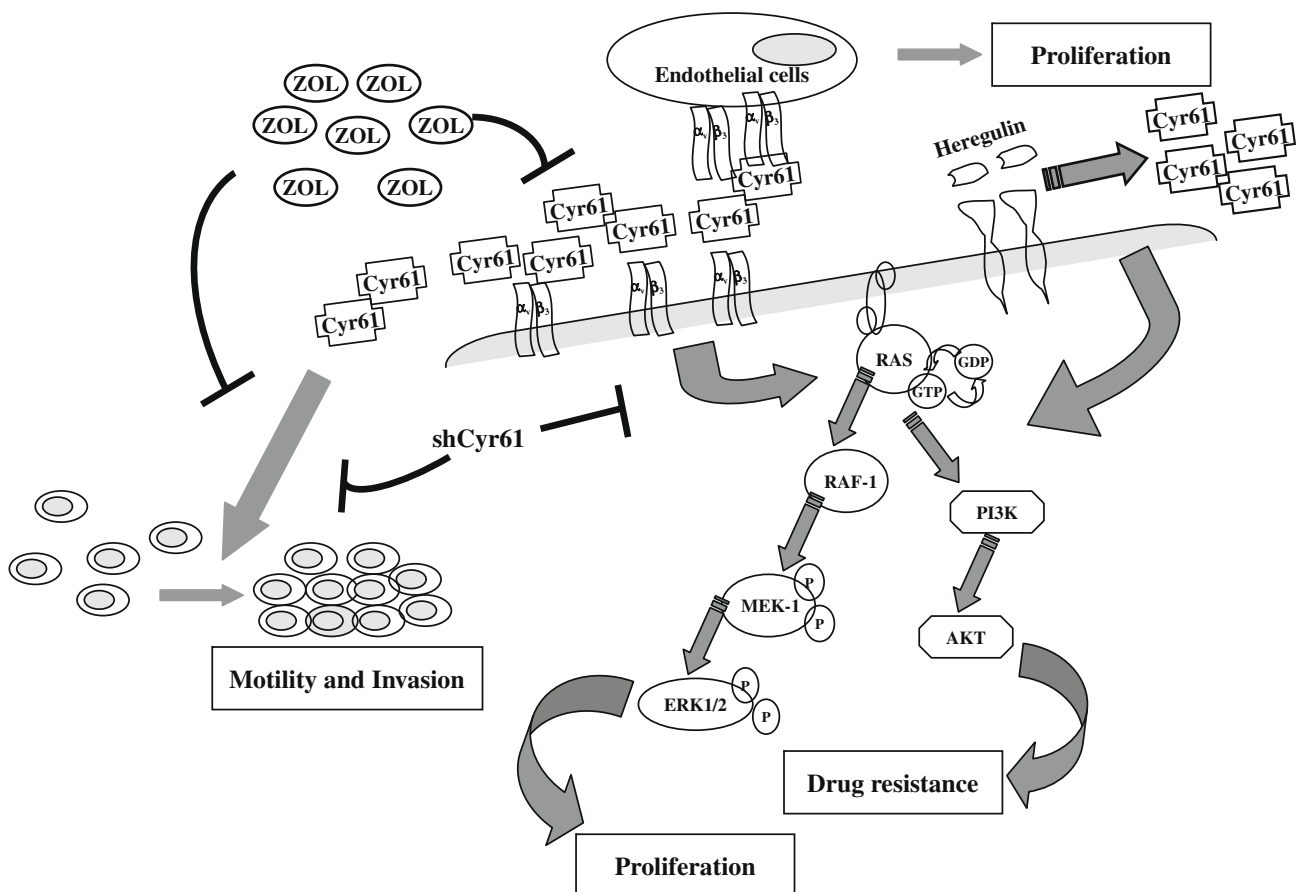
Fig. 1 – ZOL-induced downregulation of Cyr61 may predispose tumour cells towards deregulated proliferation and chemoresistance: a working model. Cyr61-activated αvβ3 integrin signalling is actively involved in cancer cell survival. Cyr61, interacting with αvβ3 integrin, induces activation of several signal transduction pathways such as the Akt- and Erk-dependent pathway thus controlling cell proliferation, apoptosis and drug resistance. Moreover, αvβ3 integrin is also directly involved in the regulation of both cell motility and invasion. Similar effects can also occur on endothelial cells surrounding Cyr61 expressing tumour cells, and this can, in turn, induce endothelial cell proliferation and angiogenesis activation. The functional blocking of αvβ3 integrin induced by either ZOL or specific siRNA raised against Cyr61 inhibits all these biological events causing a pleiotropic anti-tumour effect.

33,000 well-known human genes). The expression of the proteins encoded by the modulated genes was evaluated through western blotting analysis using specific antibodies. We found that the upregulated and downregulated genes were 73/33.000 (four genes were downregulated and 69 genes were upregulated). Among the upregulated genes, the genes coding for calreticulin gene, n-myc downstream regulated gene 1 (NDGR1) and catenin gene resulted highly upregulated with a fold-change of 11.47, 4.81 and 3.25, respectively. Among the downregulated genes, the gene coding for cysteine-rich, angiogenic inducer, 61 (Cyr61) resulted highly downregulated with a fold-change of 5.58. Cyr61 over-expression in tumour cells promotes tumour growth and vascularisation. The evaluation of the involvement of these molecules involved in angiogenesis and in differentiation of human prostate cancer cells treated with ZOL is ongoing (Fig. 1). Preliminarily, we have found the reduction of the transcriptional activity of Cyr61 promoter in ZOL-treated PC3 cells in a time-dependent manner (with a peak at 12 h). Moreover, Cyr61 protein expression was significantly decreased after exposure to ZOL. Interestingly, other inhibitors of Ras (such as R115777) or of the tyr kinase associated to EGF-R (gefitinib) or of C-Raf (BAY 43-9006) used at equitoxic concentrations did not induce or induced less effects on Cyr61 modulation when compared to ZOL. Moreover, ZOL downregulated Cyr61 and blocked the Ras-raf-1-dependent pathway. The effects of ZOL were antagonised by the addition of either farnesol or geranylgeraniol. Thereafter, we have investigated on the role of Cyr61 in apoptosis and growth regulation of PC3 cells using a shRNA for Cyr61 in order to downregulate the expression of the protein. shCYR61 enhanced growth inhibition induced by ZOL with a potentiation factor of 5, while the transfection of PC3 cells with shCYR61 alone is ineffective. The sensitisation of PC3 cells to the anti-proliferative effects of ZOL induced by CYR61 knock-down is paralleled by the inactivation of Ras, Erk and Akt. Since Cyr61 is a ligand of alphavbeta3 integrin, we have evaluated the effects of Cyr61 knock-down on motility and invasion. We have found that shCyr61 transfection induced 45% and 20% reduction of motility and invasion, respectively, but treatment of shCyr61-transfected cells with ZOL induced 75% and 30% inhibition of motility and invasion, respectively. Moreover, Cyr61 down-modulation induced by ZOL sensitises prostate cancer cells to DTX. In fact, the treatment of PC3 with DTX induced an about 2-fold increase of Cyr61. In conclusion, Cyr61 downregulation is involved in the regulation of motility processes and, indirectly, of cell growth inhibition induced by ZOL[46]. We are now evaluating the activity of blocking anti-CYR61 antibodies in inducing the potentiation of ZOL anti-proliferative effects (Fig. 1). These results could be useful in designing new therapeutical approaches in androgen-independent prostate cancer.

5. Conclusions

Based on these potential anti-cancer properties, several clinical trials have been initiated to test the combination of ZOL and other agents. The accumulated encouraging evidence indicates that ZOL is an attractive anti-cancer agent that promises to be an unexpected next exciting therapy for patients with bone tumours and metastases.

Conflict of interest statement

The authors disclose any financial and personal relationship with other people or organisations that could inappropriately influence their work.

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