Inhibition of the mevalonate pathway to override chemoresistance and promote immunogenic cell death in cancer cells: hitting two birds with one stone.

C. Riganti, M. Massaia

Inhibition of the mevalonate pathway to override chemoresistance and promote immunogenic cell death in cancer cells: hitting two birds with one stone.

ONCOIMMUNOLOGY (2013) 2

DOI: 10.4161/onci.25770

Inhibition of the mevalonate pathway to override chemoresistance and promote immunogenic cell death in cancer cells: hitting two birds with one stone

Running title: Manipulation of the mevalonate pathway in cancer with aminobisphosphonates

Chiara Riganti[1] and Massimo Massaia[2,3]

[1] Department of Oncology, School of Medicine, University of Torino, Torino, Italy; [2] Department of Molecular Biotechnology and Health Sciences, School of Medicine, University of Torino, Torino, Italy; [3] Laboratorio di Ematologia Oncologica, Centro di Ricerca in Medicina Sperimentale (CeRMS), Azienda Ospedaliera Città della Salute e della Scienza di Torino, Torino, Italy;

Corresponding author: Prof. Massimo Massaia, Divisione Universitaria di Ematologia, Azienda Ospedaliera Città della Salute e della Scienza di Torino, via Genova 3, 10126 Torino, Italy; email address: massimo.massaia@unito.it

Abstract

The mevalonate pathway is an attractive target to override multidrug resistance (MDR) and restore immunogenic cell death (ICD) in tumor cells. Recent data indicate that aminobisphosphonates are better tools than statins to manipulate the Mev pathway since they have synergistic effects on tumor cells and host immunity.

Manuscript

The final products of the mevalonate (Mev) pathway are cholesterol and isoprenoids, such as isopentenyl pyrophosphate (IPP), farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP). Isoprenoids are critical for the post-translational modification of proteins that are essential in cell growth and differentiation such as the small GTP-binding proteins Ras and RhoA.

Increasing evidences indicate that the Mev pathway is also important in multidrug resistance (MDR), a major challenge to durable tumor cell eradication by chemotherapy. The membrane transporter P-glycoprotein (Pgp) plays a key role in MDR by promoting the efflux from tumor cells of drugs such as doxorubicin (Doxo). Pgp activity in tumor cells is fostered by an accelerated Mev pathway via several mechanisms including: 1) high levels of cholesterol in the plasma membrane;[1] 2) a decreased synthesis of the endogenous Pgp-inhibitor nitric oxide mediated by GGPP-induced RhoA activation; [2] 3) enhanced HIF-1α-induced Pgp transcription induced by the activation of the RhoA/RhoA kinase and Ras/ERK1/2 signalling pathways.[3]

Interestingly, Pgp overexpression also impairs calreticulin (CRT) activity. [4] CRT translocation on the cell surface is an hallmark of immunogenic cell death (ICD), a peculiar type of cell death triggered by drugs such as doxorubicin (Doxo) which kill tumor cells and concurrently induce antitumor immune responses. CRT expression is sensed by dendritic cells (DCs) as an ‘‘eat me’’ signal, promoting tumor cell phagocytosis and cytotoxic T-cell activation.[5]

These data suggest that tumor cells that are resistant to direct chemotherapy-induced cytotoxicity also have a tendency to escape chemotherapy-induced ICD. We have recently tested this hypothesis

and showed that an accelerated Mev pathway is a common denominator bridging MDR and ICD in tumor cells.[3] Thus, inhibition of the Mev pathway is an attractive strategy to override MDR and reinstate ICD in tumor cells.

The Mev pathway can be inhibited with statins or aminobisphosphonates (NBPs). The former are specific inhibitors of β-hydroxy- β-methylglutaryl coenzyme A reductase (HMGCoAR), the rate limiting enzyme in the Mev pathway, while NBPs are specific inhibitors of FPP synthase (FPPS). NBPs are used to inhibit osteoclast activity in cancer patients and osteoporosis with zoledronic acid (ZA) being the most potent NBP currently available for clinical use.

The Mev pathway is not unique to tumor cells, and other cells with an accelerated Mev pathway activity, such as monocytes and dendritic cells (DCs), are targeted by statins such as simvastatin (Sim) or ZA eventually delivered in association with chemotherapy drugs.

Figure 1 illustrates the different scenarios that Mev pathway inhibition in tumor cells and DCs with ZA or Sim can trigger in association with Doxo. Both agents cause intracellular FPP and GGPP deprivation, downregulate Ras and RhoA activation, and decrease cholesterol production, but ZA only, by targeting FPPS downstream to HMGCoAR, increases intracellular IPP levels and induces extracellular IPP release.[6] Interestingly, IPP is very similar to the natural ligands of Vγ9Vδ2 T cells, a unique subset of unconventional T cells deeply involved in innate immune responses against microbes, stressed cells and tumor cells.[7] Several groups have shown that ZA-induced IPP accumulation in tumor cells or antigen-presenting cells (APC) such as monocytes and DCs can be exploited to intentionally activate Vγ9Vδ2 T cells and trigger immune responses against tumor cells.[8]

In MDR+ tumor cells (panel A, left), ZA interrupts Ras- and RhoA-dependent downstream signalling pathways, abrogates HIF-1α-driven Pgp expression, induces intracellular Doxo accumulation, promotes CRT exposure and restores ICD, as documented by the ability of DCs to phagocytise MDR+ cells treated with ZA and Doxo (ICD afferent arm), and to recruit anti-tumor cytotoxic CD8+ T lymphocytes (ICD efferent arm).[3] Moreover, the ZA-induced intracellular

accumulation and extracellular release of IPP facilitate the activation and immune recognition of MDR+ cancer cells by Vγ9Vδ2 T cells.[6]

In DCs (panel A, right), ZA-induced isoprenoid deprivation leads to caspase-1 activation, IL-18 and IL-1β production, and NK-cell activation.[9] ZA-treated DCs also accumulate and release high amounts of IPP leading to the concurrent Vγ9Vδ2 T-cell activation.[6] Vγ9Vδ2 T-cell activation is not detrimental to NK cells whose immune performances are improved upon interaction with activated Vγ9Vδ2 T cells.[10] Lastly, Vγ9Vδ2 T cells activated by ZA-treated DCs and tumor cells can act as cellular adjuvants and boost the afferent and efferent ICD arms. Antitumor immune responses mediated by MHC-restricted CD8+ αβ T cells are amplified by the concurrent activation of Vγ9Vδ2 T cells induced by autologous DC copulsed with ZA and tumor-associated antigens.[6] In conclusion, ZA-induced Mev pathway inhibition in tumor cells and DC can override MDR and promote the immune recognition of cancer cells by innate and adaptive immune effector cells.

The same favourable scenario is not induced by Sim (panel B) which is unable to downregulate Pgp expression.[1] Thus, Doxo accumulation in Sim-treated tumor cells is suboptimal to induce CRT exposure and ICD. Moreover, Sim does not induce any IPP accumulation in tumor cells and/or DCs, and therefore does not recruit any other immune effector cells besides NK cells. These data and the discrepancy between the doses used *in vitro* and *in vivo* may explain why statins have failed in clinical trials as chemosensitizer agents.

In conclusion, current data tip the balance in favour of Mev pathway inhibition with ZA rather than statins as the most promising strategy to override MDR, restore ICD, and promote tumor cell killing by innate and adaptive immune effector cells.

This work was supported by AIRC (MFAG 11475 to CR and IG 13119 to MM), PRIN 2010–2011 to MM and FIRB 2012 to CR.

References

1. Kopecka J, Campia I, Olivero P, Pescarmona G, Ghigo D, Bosia A, et al. A LDL-masked liposomal-doxorubicin reverses drug resistance in human cancer cells. J Contr Rel 2011; 149:196-205.
2. Riganti C, Orecchia S, Pescarmona G, Betta PG, Ghigo D, Bosia A. Statins revert doxorubicin resistance via nitric oxide in malignant mesothelioma. Int J Cancer 2006; 119:17-27.
3. Riganti C, Castella B, Kopecka J, Campia I, Coscia M, Pescarmona G, et al. Zoledronic acid restores doxorubicin chemosensitivity and immunogenic cell death in multidrug-resistant human cancer cells. Plos ONE 2013; 8:e60975.
4. Kopecka J, Campia I, Brusa D, Doublier S, Matera L, Ghigo D, et al. Nitric oxide and P-glycoprotein modulate the phagocytosis of colon cancer cells. J Cell Mol Med 2011; 15:1492-504.
5. Zitvogel L, Kepp O, Kroemer G. Immune parameters affecting the efficacy of chemotherapeutic regimens. Nat Rev Clin Oncol 2011; 8:151–60
6. Castella B, Riganti C, Fiore F, Pantaleoni F, Canepari ME, Peola S, et al. Immune modulation by zoledronic acid in human myeloma: an advantageous cross-talk between Vγ9Vδ2 T cells, αβ CD8+ T cells, regulatory T (Treg) cells, and dendritic cells. J Immunol 2011; 187:1578-90.
7. Bonneville M, O'Brien RL, Born WK. Gammadelta T cell effector functions: a blend of innate programming and acquired plasticity. Nat Rev Immunol. 2010; 10: 467-78.
8. Castella B, Vitale C, Coscia M, Massaia M. Vγ9Vδ2 T-cell based immunotherapy in hematological malignancies: from bench to bedside. Cell Mol Life Sci. 2011; 68:2419-32.

9. Gruenbacher G, Gander H, Nussbaumer O, Nussbaumer W, Rahm A, Thurnher M. IL-2 costimulation enables statin-mediated activation of human NK cells, preferentially through a mechanism involving CD56+ dendritic cells. Cancer Res. 2010; 70: 9611-20.
10. Maniar A, Zhang X, Lin W, Gastman BR, Pauza CD, Strome SE, et al. Human gammadelta T lymphocytes induce robust NK cell-mediated antitumor cytotoxicity through CD137 engagement. Blood. 2010; 116: 1726-33

Figure legend.

Figure 1. Consequences of Mev pathway inhibition in tumor cells and DCs with zoledronic acid (ZA) **(A)** or simvastatin (Sim) **(B).**

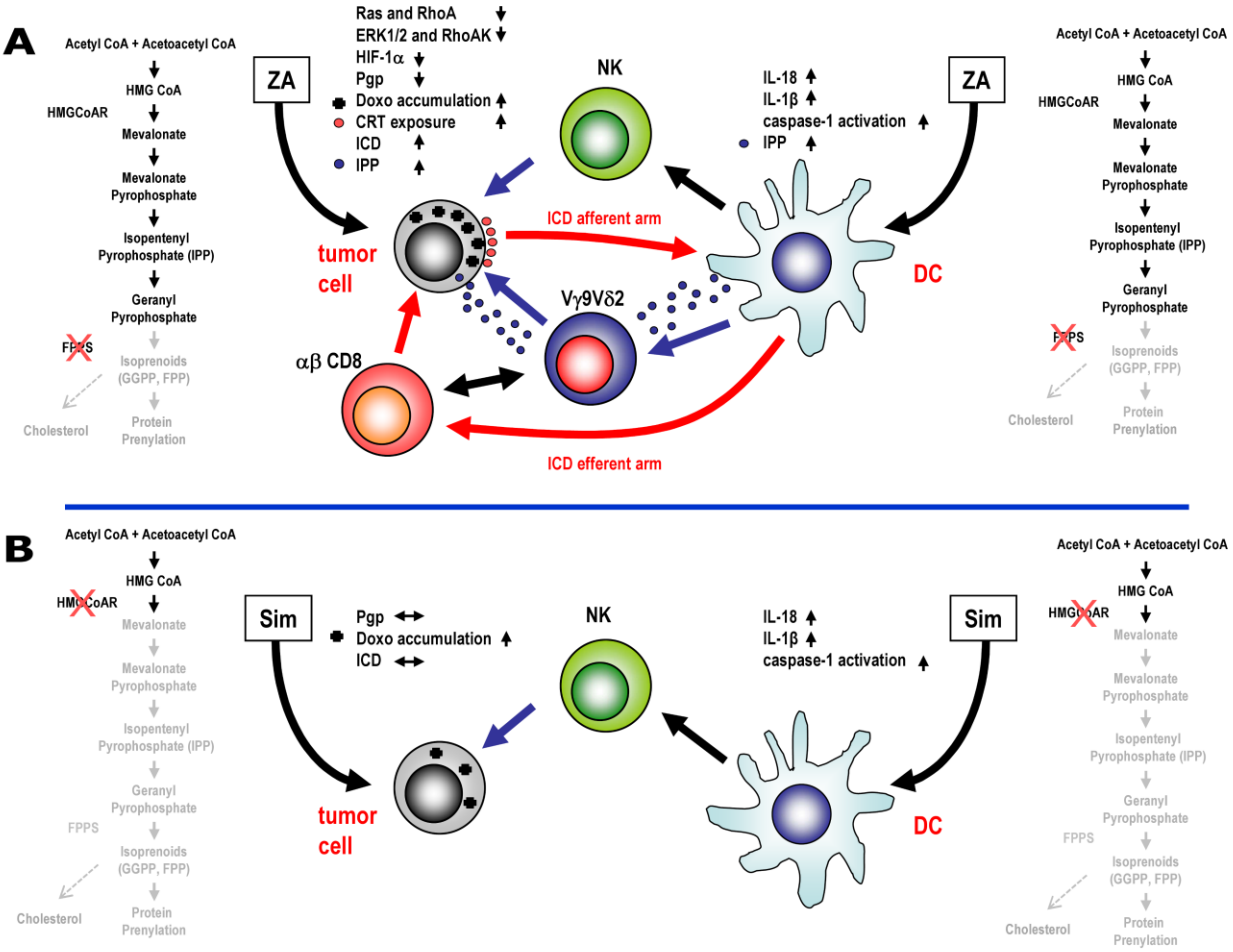
A) In tumor cells, ZA-induced FPPS inhibition results in decreased HIF-1α activity, lower Pgp expression, increased Doxo accumulation, and CRT exposure. Dying tumor cells are sensed by DCs (ICD afferent arm) and antitumor cytotoxic T cells are recruited (ICD efferent arm). ZA also induces IPP accumulation and release, leading to the activation of Vγ9Vδ2 T cells and increased recognition of tumor cells by Vγ9Vδ2 T cells.

In DCs, ZA-induced isoprenoid deprivation induces IL-18 and IL-1β production, caspase-1 activation and NK cell activation. ZA-treated DCs also accumulate and release IPP and further activate Vγ9Vδ2 T cells, which are potent adjuvants of the MHC-restricted αβ CD8+ T cells and NK cells. Thus, the afferent and efferent arms of ICD are boosted by the concurrent activation of Vγ9Vδ2 T cells.

B) Sim-induced HMGCoAR inhibition in tumor cells leads to decreased cholesterol synthesis, decreased Pgp activity and increased Doxo accumulation. However, these effects are not potent enough to significantly decrease Pgp expression, induce CRT and promote ICD. Moreover, Sim does not induce any IPP accumulation and/or release and therefore there is no recruitment of Vγ9Vδ2 T cells.

Abbreviations: CoA: coenzyme A; HMGCoA: 3-hydroxy-3-methyl-glutaryl- coenzyme A; HMGCoAR: HMGCoA reductase; IPP, isopentenyl pyrophosphate; FPP, farnesyl pyrophosphate; GGPP, geranylgeranyl pyrophosphate; FPPS: FPP synthase; HIF-1α, hypoxia inducible factor-1α; Pgp, P-glycoprotein; Doxo: doxorubicin; CRT: calreticulin; ICD: immunogenic cell death; NK, natural killer; IL, interleukin; ZA, zoledronic acid.

Figure 1


A
Acetyl CoA + Acetoacetyl CoA
HMG CoA
HMGCoAR
Mevalonate
Mevalonate Pyrophosphate
Isopentenyl Pyrophosphate (IPP)
Geranyl Pyrophosphate
FPPS
Isoprenoids (GGPP, FPP)
Cholesterol
Protein Prenylation
ZA
Ras and RhoA
ERK1/2 and RhoAK
HIF-1α
Pgp
Doxo accumulation
CRT exposure
ICD
IPP
NK
IL-18
IL-1β
caspase-1 activation
tumor cell
ICD afferent arm
DC
Vγ9Vδ2
αβ CD8
ICD efferent arm
B
Sim
Pgp
Doxo accumulation
ICD