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An iron hand over cancer stem cells

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ABSTRACT

The paradigm of cancer stem cells (CSCs) defines the existence of cells exhibiting self-renewal and tumor-seeding capacity. These cells have been associated with tumor relapse and are typically resistant to conventional chemotherapeutic agents. Over the past decade, chemical biology studies have revealed a significant number of small molecules able to alter the proliferation of these cells in various settings. The natural product salinomycin has emerged as the most promising anti-CSC agent. However, an explicit mechanism of action has not yet been characterized, in particular due to the pleiotropic responses salinomycin is known for. In this punctum, we describe our recent discovery that salinomycin and the more potent synthetic derivative we named ironomycin sequester lysosomal iron. We found that these compounds, by blocking iron translocation, induce an iron-depletion response leading to a lysosomal degradation of ferritin followed by an iron-mediated lysosomal production of reactive oxygen species (ROS) and a cell death pathway that resembles ferroptosis. These unprecedented findings identified iron homeostasis and iron-mediated processes as potentially druggable in the context of CSCs.

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Cancer stem cells represent a small fraction of solid tumors in most cases and are typically refractory to conventional treatments. The ability of these cells to maintain stemness is highly dependent on the tumor microenvironment and the status of neighboring cells. Thus, studying CSC physiology using cells isolated from tumors is least relevant, making the identification of small molecules effective against the proliferation of these cells most challenging. As a means to identify druggable targets in CSCs, several groups have engineered cell lines that exhibit traits of CSCs (e.g., high levels of CD44 cell surface marker, low levels CD24, ALDH1/aldehyde dehydrogenase 1 activity, the capacity to form tumor-spheres under nonadherent cell culture conditions and to seed tumors in vivo, making it possible to evaluate the activity of small molecules in large scale. In particular, normal breast human mammary epithelial cells have been transformed with TERT/hTERT, SV40 and $HRAS^{G12V}$, stably repressing the cell adhesion protein CDH1/E-cadherin, a characteristic of mesenchymal cells. Previous work had led to the identification of the ionophoric natural product salinomycin that selectively kills these cells over nonstem isogenic cells. It was hypothesized that the anti-stem activity is linked to the capacity of salinomycin to transport sodium and potassium ions through lipid membranes thereby altering membrane potentials and initiating cell death. Additionally, salinomycin was suggested to promote or inhibit autophagic flux, to induce ER stress and mitochondrial outer membrane permeabilization as well as genomic instability among many other defects.

Whereas salinomycin appears to be pleiotropic by nature, we hypothesized that a single event operating upstream and independently of alkali metal transport may be at work. To investigate this further, we developed a surrogate salinomycin drug, we termed ironomycin, that is at least 10-fold more potent than salinomycin against CSCs in vitro and in vivo. We found that ironomycin effectively depletes the population of CSCs in patient-derived xenograft models resistant to the landmark drug docetaxel. Remarkably, we showed that the ionophoric property of these drugs was marginal at effective doses. Furthermore, ironomycin was designed to be detectable in cells by high-resolution photon microscopy. More specifically, we used click-chemistry to chemically label the alkyne-containing small molecule in cells, allowing us to detect the subcellular distribution of ironomycin. By doing so, we discovered that, contrary to expectations, this salinomycin derivative did not exhibit a diffuse cytosolic pattern but instead quantitatively accumulated in the lysosomal compartment. Unlike other lysosomotropic small molecules such as artesumycin that enter cells by means of endocytosis, we found that ironomycin freely diffuses through the plasma membrane before reaching the

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lysosomal compartment, in strong agreement with the amphiphilic properties of this drug.

Further analyses indicate that salinomycin and ironomycin prevent translocation of iron from the lumen to the cytosol and consequently initiate an iron-depletion response. This response is characterized by increased levels of IREB2/IRP2 (iron responsive element binding protein 2) and TFRC/CD71 (transferrin receptor) along with a rapid degradation of the iron storage protein ferritin. Interestingly, ferritin degradation is predominantly mediated by the lysosomal protease CTSB (cathepsin B), and ferritin relocalization to the lysosomal compartment can also be observed. Mechanisms through which ferritin is delivered to lysosomes remain to be fully characterized. Nevertheless, iron loading in lysosomes upon treatment with salinomycin or ironomycin is directly evidenced using RhoNox-1 staining, an iron(II)-specific probe, providing the first evidence of such an effect mediated by a small molecule. This property rationalized the pleiotropic effect of salinomycin observed in other organelles and represents a unifying mechanism. Furthermore, we used NMR spectroscopy to show that salinomycin and ironomycin can interact with iron(II) in vitro, confirming the contention that these drugs sequester the metal as a result of a direct interaction.

We found that treated cells rapidly accumulate lysosomal ROS as a result of iron overload and the well-established reactivity of this metal toward molecular oxygen and hydrogen peroxide (e.g., Fenton chemistry). Consistent with our findings, cell death induced by salinomycin or ironomycin is prevented by a CTSB inhibitor, a ROS scavenger and the strong iron chelator deferoxamin. It is noteworthy that tight chelators can abolish Fenton chemistry by poisoning the metal, indicating that in contrast to deferoxamin, salinomycin and ironomycin are looser binders capable of interacting with the metal and allowing for the production of ROS. Presumably as a result of ROS production, we can detect lysosome membrane permeabilization, lipid peroxidation and decreased endogenous levels of the ROS scavenger glutathione, which is consistent with the induction of ferroptotic cell death. Importantly, the pronounced effect of ironomycin against CSCs is linked to higher levels of iron in these cells and upregulated iron regulatory proteins including active CTSB and TFRC/CD71 compared with isogenic non-stem cancer cells. These findings directly implicate iron and iron homeostasis in the maintenance of CSCs. Altogether, our findings pave the way toward an unprecedented therapeutic strategy to eradicate CSCs by targeting the lysosomal pool of iron.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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