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Review

The role of proton dynamics in the development and maintenance of multidrug resistance in cancer

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ABSTRACT

With a projected 382.4 per 100,000 people expected to suffer from some form of malignant neoplasm in 2015, improving treatment is an essential focus of cancer research today. Multi-drug resistance (MDR) is the leading cause of chemotherapeutic failure in the treatment of cancer, the term denoting a characteristic of the disease-causing agent to avoid damage by drugs designed to bring about their destruction. MDR is also characterised by a reversal of the pH gradient across cell membranes leading to an acidification of the outer milieu and an alkalinisation of the cytosol that is maintained by the proton pump vacuolar-type ATPase (V-ATPase) and the proton transporters: Na^+/H^+ exchanger (NHE1), Monocarboxylate Transporters (MCTs), Carbonic anhydrases (CAs) (mainly CA-IX), adenosinetriphosphate synthase, Na^+/HCO_3^- co-transporter and the Cl^-/HCO_3^-exchanger. This review aims to give an introduction to MDR. It will begin with an explanation for what MDR actually is and go on to look at the proposed mechanisms by which a state of drug resistance is achieved. The role of proton-pumps in creating an acidic extracellular pH and alkaline cytosol, as well as key biomechanical processes within the cell membrane itself, will be used to explain how drug resistance can be sustained.

1. Multi-drug resistance (MDR)

In order to introduce the subject we will, firstly, discuss the current representation regarding MDR mediated by active membrane drug pumps and, in a second part, review the many hidden paradoxes behind the single notion of drug pumping to explain MDR. This will allow a smooth and natural introduction of other tumouregenic elements such as the pH gradient across the membrane and the alteration of the physical properties of the membrane.

1.1. MDR mediated by drug pumps

Multi-drug resistance (MDR) is characterised by the development of resistance to an anticancer drug, which is then accompanied by resistance to other structurally and pharmacokinetically unrelated drugs. Ultimately, MDR describes the failure of a diverse range of drugs to reach and/or act on their targets [1], which include DNA [2], RNA [3] and tubulin [4]. The phenomenon typically follows one of two pathways; either as a pre-existing phenomenon discovered after metastatic presentation, or as a metastatic recurrence following treatment of a primary tumour [5]. The challenge of MDR has confounded scientists and clinicians for many years, with a definitive solution remaining elusive. Multiple theories have been postulated regarding the conferment of MDR, implicating the P-glycoprotein (Pgp) coded by the MDR1 gene (an ATP-binding cassette [ABC] transporter). Studies have revealed that Pgp relies on the actin cytoskeleton for its localisation in lipid rafts on the cell membrane thereby facing drugs influx and probably counteracting uptakes [6,7]. In this context of membrane location mediated by actin, the interaction between ezrin and Pgp is thought to play a pivotal role in conferring the tumour cells a metastatic phenotype [8–10]. The action of Pgp as a drug efflux pump to such therapies as paclitaxel, adriamycin, docetaxel and daunorubicin [11] has led to the development of chemosensitising agents including verapamil, cyclosporine and quinine which focus on the inhibition of this protein, both competitively and noncompetitively [12]. The discovery of multi-drug resistance associated proteins (MRP), such as ABCG2 (mitoxantrone resistance protein, MXR), has widened the therapeutic scope for the inhibition of alternative efflux pumps which often share some structural similarity with Pgp, as is exhibited by the MRP1-encoded ABCC1 [12,13]. However, Pgp expression appears not to be a prerequisite of the MDR phenotype – another demonstration of the heterogeneity of tumours – and De Milito and Fais [14] concluded that '…it does not seem that ABC transporters have a key and direct role in the intrinsic resistance of tumours to anticancer

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drugs'. Although the evidence for the presence and role of drug efflux pumps in many cases of MDR is irrefutable, it has been shown that an intracellular alkaline shift alone is sufficient for the failure of accumulation of intracellular chemotherapeutic agents within the appropriate compartments of cells [15] accompanied by increased drug efflux and decreased cytosolic accumulation [16]. These data have resulted in a shift of the concepts used and related therapeutic goal to combine targeting both Pgp function and pH changes in cancer. In addition, the introduction of pH in MDR has opened the door to new "synthetic theories" aiming at understanding MDR as a whole and not focused only on Pgp-like drug transporters.

A quick review of paradoxes behind the single use of Pgp theory in MDR will now highlight the need for new synthetic theories involving other tumouregenic parameters, especially the pH.

1.2. Paradox one: the drug-pumped-to-ATP-consumed ratio

It was in 1973 that Dano Keld suggested that the mechanism of resistance was due to an outward efflux [17]. This hypothesis clearly gained credance when three years later P-glycoprotein (Pgp) was identified by Juliano and Ling as the membrane protein over-expressed in MDR cancer cells that actively extrude membrane amphipathic drugs [18]. Since then many biological, biochemical and structural studies have been carried out on this family of ABC transporters. To summarize, a conformational change in the structure of Pgp upon ATP binding allows access from the lipid bilayer inner leaflet to the internal cavity of volume ~6000 A^3 [19–23]. Drug binding to Pgp is more sensitive to ATP binding rather than hydrolysis, and two ATP molecules need to be bound on Pgp to allow its full activation [23–28]. The use of crystallography methods and basic biology found that the turnover rate of Pgp ATPase is in the range of ~1–15ATP/s [29–31] with a near stoichiometric substrate transport to ATP hydrolysis ~2ATP/drug, reviewed in [32].

At the molecular level everything sounds fine but what remains unclear however is the low efficacy of Pgp in reconstituted systems. The apparent stoichiometry of the hypothesised ATP coupled active drug transport, i.e. the number of ATP molecules hydrolysed per drug transported, can be enormous (calculated to be up to ~36,000 ATP/drug in reconstituted proteo-liposomes) [33–35]. This suggests that whilst consuming ATP, Pgp does not necessarily lead to drug extrusion. It seems therefore that Pgp-like transporters oscillate between open and close conformations without involving and transporting drugs. Although the history of biology (and evolution in particular) taught us that biological systems do not need to be fully efficient to keep their robustness, it is notable nonetheless that if Pgp was inefficient MDR would not be a problem in clinical oncology. Two paths are now available, either Pgp and relatives are not involved in MDR at all (that is unlikely to be the case) or something else must help Pgp and relatives to gain enough efficacy for MDR to be noticable by clinicians.

1.3. Paradox three: the role of drug molecular weight (MW) in drug resistance

Today, it is suggested that the ability of many drugs to bind the internal cavity of Pgp is linked to the number of potential binding sites available on the wall of the internal cavity composed of hydrophobic, aromatic, polar and charged amino acid residues [19]. Altought the later statement is sound from a biochemical point of view, it is important to note that the MW of drugs (namely their size or volume) is known to be a strong predictor of MDR levels in Pgp expressing cells [36–38]. This point was first demonstrated in 1970 [36]. The date is important here as this seminal study on drug resistance comes three years before Dano Keld's "vacuum cleaner" hypothesis (Dano, 1973) and six years before the discovery of Pgp [18]. So albeit the notion of drug pumping was inexistent at the time (1970), the drug MW was the main parameter describing MDR then. Why this type work based on drugs MW was not carried forward is not clear but what is remarkable however, is that decades later the pharmaceutical industry discovered that the MW of drugs is indeed paramount for their systemic delivery (bioavailability) and largely responsible for attrition [39]. From the pharmaceutical point of view, the bioavailability of a drug depends also on its ability to cross the multiple membrane layers present in a body (i.e. cells) and, accordingly, it was demonstrated that lipid bilayer membranes do indeed play a fundamental role in drug bioavailability based on their MW [37,40]. The fundamental reason behind this is related to the biomechanical interaction between the drug volume and the surface tension of the cell membrane namely the physical packing of lipids in either leaflet of the cellular membrane (controlled by cells themselves).

So maybe without noticing it, Bielder and Rhiem discovered in 1970 [36] a fundamental Law in basic drug delivery [37,40].

1.4. Paradox three: the lack of specificity

As stated by the term used namely "multi drug resistance", a single transporter should be able to transport many different drugs not related structurally and chemically. Although the molecular model of Pgp has permitted a relatively simple representation of MDR in agreement with the usual concepts issued from the field of biochemistry, how a single protein can expel structurally different drugs is still poorly understood. Indeed, "controversy remains over how P-gp recognizes hundreds of different hydrophobic drugs and pump them out of the cell…" [41]. Beyond this last remark, there is something far more significant and important at stake: the Pgp-mediated MDR model does not conform to the fundamental notion of specificity and seems to challenge the roots of biochemistry. This conceptual issue was exposed early and very clearly by Paul Roepe: "…MDR cells are resistant to, and/or exhibit decreased retention of, literally hundreds of different hydrophobic compounds that are structurally divergent… Membrane transporters, like soluble enzymes, are exquisitely substrate-specific…If transporters were not specific, the cell would eventually become a high entropy chaotic mess…[as there are] no structural molecular motifs common to all the many different agents to which MDR cells are resistant…MDR protein is a very unusual enzyme with extraordinarily broad substrate recognition capabilities; that is, it violates the law of enzyme specificity" [35]. Given the paramount importance of the notions of "specificity" or "affinity" in classical biochemistry there was an obvious need to redefine Pgp efficiency.

It is common to define the binding-affinity as the likelihood of drug and transporter interacting upon meeting and in this case the interaction energy becomes a fundamental variable. However there exist chemical reactions that are relatively inefficient and one way to increase the rate of products formed is to raise the temperature. By doing so it is not the interaction energy that is affected but the rate of collisions between chemicals that is increased. By increasing collision rates the chance of a product being formed increase as well.[1] Random processes have been studied for more than a century, and it is now well established that the mathematical properties of Brownian diffusions are fully dependent on the dimensions of space. In particular, there is one theorem, known as Polya's Theorem, that states that portions of space are always left unvisited (whatever the visitation time considered) if the Brownian particle diffuses in dimensions higher than 2 and that, conversely, in dimensions smaller than or equal to 2, all the space will be visited possibly more than one time over a long enough period of time, reviewed in [42]. Recalling that the MW of drugs is important and involved in their residency time in membrane (of course function of the membrane physical properties), the larger the drug the better to improve Pgp-mediated

[1] Likewise, one has more chance of winning the national lottery if we buy more than one ticket.