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Cell-derived microvesicles and antitumoral multidrug resistance

Les microvésicules et la résistance multidrogue antitumorale

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ABSTRACT

Antitumoral chemotherapeutic treatments are often impaired by innate or acquired multidrug resistance (MDR). After four decades of MDR research, having underlined its complexity, new knowledge about the mechanisms of tumor resistance to antineoplastic drugs is a prerequisite for improving chemotherapy. Following our observations with a non-pathogenic eukaryotic microorganism, *Dictyostelium discoideum,* I suggest that MDR in tumor cells might be the consequence of a detoxification mechanism, mediated by cell-derived microvesicles. Recently published observations with tumoral human cells support this hypothesis. First, these cell-derived vesicles might impair chemotherapeutic efficiency of many structurally-different antineoplastic agents by preventing them to reach their intracellular target, followed by their expulsion outside the tumor cells, as observed for *Dictyostelium* cells. Secondly, besides their newly recognized function of intercellular communication, the cell-derived vesicles might also act as intercellular transporters of multidrug resistance proteins. Experiments are suggested for checking the hypothesis of cell-derived vesicles mediating multidrug resistance.

Mots clés :
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RÉSUMÉ

L'échec de la chimiothérapie antitumorale est souvent la conséquence d'une résistance multidrogue (MDR) innée ou acquise. Après quarante ans de recherche, la complexité de cette résistance est un fait avéré. Mieux élucider les mécanismes de résistance tumorale aux agents antitumoraux demeure donc une exigence pour améliorer l'efficacité de la chimiothérapie. D'après nos observations sur un microorganisme eucaryote non pathogène, *Dictyostelium discoideum,* je suggère que la résistance multidrogue des cellules tumorales pourrait résulter d'un mécanisme de détoxification cellulaire médié par des microvésicules externalisées par les cellules. Cette hypothèse est confortée par la publication d'observations récentes sur des cellules tumorales. Ces microvésicules pourraient affecter l'efficacité de la chimiothérapie en empêchant les médicaments antitumoraux d'atteindre leur cible intracellulaire. Elles pourraient également jouer un rôle actif de transporteurs intercellulaires des protéines de résistance multidrogue et propager ainsi cette capacité de résistance. Des expériences sont suggérées pour tester l'hypothèse d'une résistance multidrogue médiée par des microvésicules.

1. Introduction

Antitumoral chemotherapeutic treatments are often impaired by innate or acquired multidrug resistance

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(MDR). The ATP Binding Cassette (ABC) transporters have been recognized, as being involved in the process of expelling chemotherapeutic drugs from tumor cells [1,2], but four decades of MDR research have underlined its complexity. Multidrug resistance is also a property of bacteria, which have many transporters to get rid of different endogeneous or exogeneous compounds [3]. In human cells, as much as 48 ABC transporters genes are encoded [2,4]. At least 12 of them should be involved in resistance to antineoplastic drugs. The first most studied MDR is mediated by the so-called P-glycoprotein (P-gp) (for review see [1,2,5]). However, P-gp was early recognized as a "double-edged sword", also acting on normal cells [6]. Thus, a first question deals with the specificity of ABC transporters in tumor cells, relative to normal cells. Nevertheless, the most puzzling point is the observation that the P-gp pump is efficient against a huge number of compounds, lacking any common structural features or properties. No clear explanation for the mechanism of this non-specificity has yet been provided.

After the predominance of P-gp mediated MDR during two decades, other non-P-gp mediated MDR mechanisms were evidenced, like the one mediated by the multidrug resistance-related protein (MRP), or the lung resistance protein (LRP). Clinical outcome in cancer was then predicted by comparison of the relative expression of these three proteins [7], but no clear general prognostic value could thus be outlined.

Unravelling the mechanisms of tumor resistance to antineoplastic drugs is a prerequisite for improving chemotherapy, which remains a major goal of cancer biologists [2,4]. The new knowledge brought about by our results on a primitive eukaryotic model, *Dictyostelium discoideum* [8,9], allows us to suggest that multidrug resistance in tumor cells might mainly be the consequence of a detoxification mechanism, mediated by cell-derived vesicles.

2. A new multidrug resistance mechanism in *Dictyostelium discoideum*

In 1999, *D. discoideum* was chosen by the NIH (USA), as a non-mammalian model for biomedical researches (http://www.nih.gov/science/models/).

As related in our first paper [8], *D. discoideum* cells are highly resistant to the DNA vital stain Hoechst 33342 (HO342). These cells are also resistant to many structurally unrelated xenobiotics, such as the carcinogenic hydrocarbon benzo(a)pyrene, the antitumoral antibiotic daunorubicin, the mitochondrial vital stain Rhodamine 123 and a carbocyanine dye, JC-1. All these xenobiotics are known substrates of the P-gp, a 170-kDa protein, mediating classical multidrug resistance.

We first demonstrated, by Western blot analysis with the anti-P-gp monoclonal antibody (mAb) JSB-1, the presence of a constitutive P-gp in *D. discoideum* cells [10]. This was confirmed by flow cytometry analysis using two other anti-P-gp mAbs, C219 and MRK-16. However, despite the presence of P-gp, the classical P-gp-mediated multidrug resistance appeared non-functional in *D. discoideum* cells.

Searching for a new resistance mechanism to explain the high endogeneous resistance of *D. discoideum* cells, we observed that, when assays of vitally staining the cells with the DNA-targeted dye, HO342 were performed, a fluorescent material was released in the culture medium. The spectral characteristics of the fluorescence allowed us to identify HO342 as the emitting compound [8,9]. After concentration, the vesicular nature of this fluorescent extracellular material was revealed by electron microscopy and phospholipid identification. Nucleic acids were also found associated with these vesicles [8]. The new extracellular organelles were further characterized by cryo-electron microscopy and protein analysis [11]. Thus was evidenced in *D. discoideum* cells, an unknown multidrug resistance mechanism [8], sustained by a detoxification process, by means of which the cells get rid of different structurally unrelated xenobiotics. Moreover, these *Dictyostelium* extracellular vesicles were able to transfer their drug content to human cell lines, such as the erythroleukemic K562 cells [9] and tumoral HeLa cells [11]. This "Trojan-horse" capacity of *Dictyostelium* extracellular vesicles was accredited for therapeutic non-viral drug delivery[1], whereas their immunogenicity was checked by a first in vivo study, showing a specific antibody response, but no pyrogenic response nor any inflammation, as measured by five pertinent cytokines (study performed by *Genosafe*, Evry, France) [11].

3. Hypothesis of a new multidrug resistance mechanism in mammalian cells

Although being a very primitive eukaryotic ancestor of mammalian cells [12], *Dictyostelium* was recently recognized as a model for human disease [13]. This model turned valuable for the fundamental study of many unrelated human pathologies, such as pathogen-host interactions [14,15], pathobiology of cell motility [16], analyses of lissencephaly-related proteins [17], mechanisms involved in sensitivity to cisplatin and other anticancer drugs [18], signaling in bipolar disorder [19], lysosomal and trafficking diseases [20] and mitochondrial disease [21].

Taking into account both the above consideration and the amazing continuity in many biological processes from primitive to higher organisms, we suggest a new hypothesis about the mechanism of multidrug resistance in mammalian cells. The newly observed vesicle-mediated detoxification mechanism in *D. discoideum* cells [8,9,11,22] might also be involved in mammalian cells, and explain, at least partially, the failure of antitumoral chemotherapy.

Already in 2003, Shedden et al. mentioned that, when treated with doxorubicin or other antitumoral compounds, many different tumor cells release vesicles, inholding the antitumoral compound [23]. Some recent

[1] Tatischeff I; Alfsen A.; Lavialle F., Extracellular vesicles from non-pathogenic amoeba useful as vehicle for transferring a molecule of interest to an eukaryotic cell. *(DRITT-UPMC)* Patent European priority N° 03 291 752 07/15/2003 ([European Patents in Danemark, Deutschland, France, Great Britain, Italy, Netherlands and Spain), US Patent and Pending Patent in Canada]).

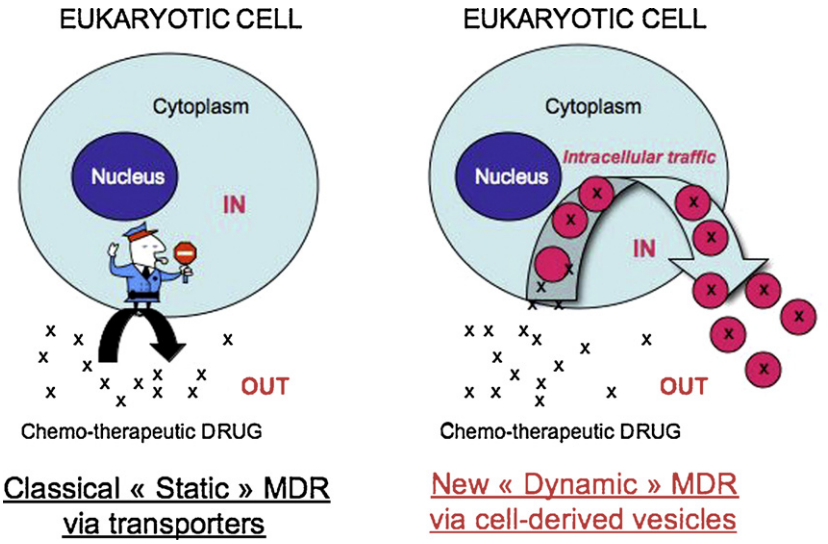

Fig. 1. Schematic comparison of the new vesicle-mediated multidrug resistance mechanism with the classical one.

clinical observations fit with our suggestion and underline the importance of membrane vesicles in tumorigenesis [24]. Very recently, Goler-Baron and Assaraf observed extracellular vesicles mediating multidrug resistance between two attached MCF-7/MR cells [25]. Fig. 1 depicts schematically the suggested new "dynamic" multidrug resistance (MDR), mediated via cell-derived vesicles, originating from the endocytic intracellular traffic. The classical multidrug resistance (MDR), mediated via known transporters, like P-glycoprotein, multidrug resistance proteins (MRP) (and others), corresponding to a "static" mechanism preventing many therapeutic drugs to enter the cells, is shown for comparison.

4. Suggested experiments to check this hypothesis in human cells

Two lines of experiments are proposed to check the hypothesis that cell-derived vesicles are also drug transporters in human cells.

First, our observation that *Dictyostelium* cells are resistant to the vital staining of their nuclei by HO342 [8,9] could be linked with the fact that the detection by flow cytometry of the few hematopoietic stem cells in a cell population has also long been based on their high resistance to the vital staining of their nuclei by HO342 [26,27]. Therefore, it would be worth searching whether a stem cell population, grown in the presence of HO342 should depict the same vesicle-mediated detoxification mechanism, as the one, which we evidenced in *D. discoideum* cells. Very recently, tumors where suggested to harbour stem cells [4] and it would, therefore, be interesting to evidence such a multidrug resistance mechanism in stem cells.

On the other hand, with regard to non-solid tumors, the human erythroleukemia K562s cell line, normally sensitive to chemotherapy, can become a resistant K562r cell line by an appropriate long term drug treatment [28]. Up to now, this resistance was thought to be mainly mediated by the P-glycoprotein. K562 cells are also known to constitutively expel "exosome-like" vesicles in their extracellular medium [29]. We wonder whether these vesicles could also be involved in a multidrug resistance mechanism, like *Dictyostelium* vesicles, which turned out to be both constitutive without drug, and involved in drug detoxification [8]. These two cell lines, K562s and K562r, would, indeed, be a good choice to check the general occurrence of a multidrug "vesicle-mediated" resistance mechanism. Vesicles could be concentrated from both extracellular growth media, following incubation of the two cell lines with a fluorescent P-gp substrate, like HO342, and P-gp expression should be compared in the vesicles originating from K562s and K562r cells, respectively. If P-gp might be transported by vesicles, originating from K562r cells, it might, possibly, also be transferred to K562s cells by means of these vesicles. This could sign a way of propagating multidrug resistance both in vitro and in vivo, as already observed in the vesicle-mediated propagation of a few tumors [24].

5. Conclusion

D. discoideum has already shown its interest as a model for human disease [13–21]. It could as well be thought as a model for unraveling the mechanisms of multidrug resistance, still severely impairing the antitumoral chemotherapeutic treatments.

Based on our previous results, which evidenced a new vesicle-mediated multidrug resistance in *D. discoideum* cells [8,9,11], the observed detoxification mechanism is suggested to be a quite general eukaryotic process, also implied in antitumoral multidrug resistance. Two lines of experiments with human cells are proposed to check this hypothesis.

Moreover, the newly recognised biological importance of cell-derived vesicles in intercellular communication[2]

[2] International Workshop on Exosomes (IWE) 2011, January 19–22, 2011, institut Curie, Paris, France.

[30–32], as well as their importance in tumorigenesis [24,33] could also be advantageously further questioned by using *Dictyostelium* as a model.

Disclosure of interest

The author declare that he has no conflicts of interest concerning this article.

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