TITLE:VIOLACEIN-LADED *Salmonella* Typhimurium OUTER-MEMBRANE VESICLES EXERT ANTI-TUMOR ACTIVITY

AUTHORS: PEREZ JORGE, G.[1]; GONTIJO, M.T.P.[1]; REZENDA, C.P[2]; OLIVEIRA, B.T.M[2]; BILSLAND, E.[2]; ALMEIDA, F.B.R.[3]; BROCCHI, M.[1]

INSTITUTION: 1.DEPTO. GENÉTICA, EVOLUÇÃO, MICROBIOLOGIA E IMUNOLOGIA E 3. DEPTO DE BIOLOGIA ESTRUTURAL E FUNCIONAL, INSTITUTO DE BIOLOGIA, UNIVERSIDADE ESTADUAL DE CAMPINAS, CAMPINAS, SP (CIDADE UNIVERSITÁRIA ZEFERINO VAZ, 255 – BARÃO GERALDO, CEP 13083-970, CAMPINAS – SP, BRASIL; 2. DEPTO DE BIOQUÍMICA E IMUNOLOGIA, FACULDADE DE MEDICINA DE RIBEIRÃO PRETO, UNIVERSIDADE DE SÃO PAULO, RIBEIRÃO PRETO, SP(AV. BANDEIRANTES, 3900 - CAMPUS DA USP, CEP 14049-900, RIBEIRÃO PRETO - SP, BRASIL.

ABSTRACT:
Violacein, a purple-colored pigment isolate from different environmental bacteria such as *Chromobacterium violaceum* has a wide spectrum of biological actions, including antitumor activity, which suggests a future clinical application in cancer therapy. An efficient and safe drug delivery system is needed for the clinical use of violacein in cancer therapy. Recent studies have shown that outer membrane vesicles (OMVs) derived from Gram negative bacteria can be used as efficient nanocarriers of anticancer drugs. OMVs are spherical nanometric proteoliposomes (20-250nm), produced naturally and secreted by gram-negative bacteria. OMVs can fuse with host cells membrane and release their content into the cytosol. This study was conducted to investigate the antitumor activity of OMVs derived from *Salmonella enterica* Typhimurium 14028 (*S.* Typhimurium) loaded with violacein.One difficulty of studies with OMVs is the low yield of OMVs during isolations; to overcome this, we constructed a mutant with a hypervesiculated phenotypeby the deletion of two genes that code for cell envelope proteins, engineered the strain *S.* Typhimurium Δ*tol*RΔ*tol*A. This strain and its parent were transformed with the plasmid pBATvioABCDE, which carries the operon for the synthesis of violacein. Isolation of OMVs was performed by the ultracentrifugation method from cultures of *S.* Typhimurium, *S.* Typhimurium pBAT*vioABCDE*, *S.* Typhimurium Δ*tol*RΔ*tol*A and *S.* Typhimurium Δ*tol*RΔ*tol*A pBAT*vioABCDE*. The OMVs were characterized by nanoparticle tracking analysis (NTA) and by transmission electron microscopy (TEM). OMVs were quantified based on total protein content (Bradford). Finally, the antitumor activity of OMVs was analyzed in vitro in melanoma cells (B16F10). OMVs purified from bacteria carrying pBAT*vioABCDE* were purple, suggesting the presence of violacein. We confirm that *tol*R and *tol*A mutations in *S.* Typhimurium significantly increase OMVs production, as determined by protein and NTA (particles/mL). TEM and NTA did not reveal significant differences between the size of the double mutant and the wild type strain. Our results showed that violacein-loaded OMVs significantly decrease melanoma cell viability by more than 90% (10µg/mL). Our findings suggest that OMVs can be used as nanocarriers and that violacein-loaded OMVs have the potential to be used as antitumor therapy.
Keywords: violacein, vesicles, OMVs, melanoma, hypervesiculation, *Salmonella* Typhimurium.

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