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Prolonged intracellular accumulation of light-activatable nanoparticles in leukemic cells allows remote activation

The differentiation of leukemic cells is a therapeutic platform very often used in the clinic to eradicate blood cancers, being the concentration of the inductive agent and the spatio-temporal control of its application very important variables for the success of the therapy[1]. Induction of leukemic cell differentiation by RA is a therapeutic strategy that has been used with great success in the treatment of acute promyelocytic leukaemia (APL)[2, 3]. RA activates nuclear RA receptors (RARs) that induce cell growth arrest and differentiation[4]. Despite its clear therapeutic efficacy, approximately 25% of patients receiving RA will develop serious complications including the "differentiation syndrome"[5], and thus the need for more effective formulations to deliver RA into leukemic cells while preventing RA side effects. In addition, leukemia-maintaining cells that resist to therapy reside in microenvironmental niches at the bone marrow that are difficult to reach by conventional therapy[6]. Therefore, news strategies are required to tackle this problem.

NPs that disassemble in response to light[7-9] might address both issues. Recent light-activatable NPs have been reported to target solid tumors based in the accumulation of the NPs in tumor vasculature after intravenous injection[10]. Yet, such approach is not extensive to leukemias. The hypotheses of the current work are: (i) light-activatable NPs containing RA might be a more effective strategy to differentiate leukemic cells because they release high and more effective concentrations of RA in a short period of time (minutes range) after NP disassembly, and (ii) light-activatable NPs containing RA accumulated in the cytoplasm of leukemic cells might offer a unique opportunity to differentiate remotely these cells in leukemic niches at the bone marrow, which in turn might interfere with the differentiation profile of the leukemia-maintaining cells by paracrine factors.

Here, we describe light-activatable polymeric NPs that are very effective in accumulating in the cytoplasm of leukemia cells and to induce cell differentiation either *in vitro* or *in vivo* after light activation. To prepare light-activatable polymeric NPs, poly(ethyleneimine) (PEI) was initially derivatized with 4,5-dimethoxy-2-nitrobenzyl chloroformate (DMNC), a light-sensitive photochrome. PEI was selected as initial NP block because it facilitates the cellular internalization of NPs and subsequent escape from endosomes[11, 12], while

DMNC was selected because responds rapidly to light and the degradation products are relatively non-cytotoxic[13]. PEI-DMNC was then added to dextran sulfate (DS) to form NPs by electrostatic (PEI:DS) and hydrophobic (DMNC:DMNC) interactions. To stabilize the NP formulation, zinc sulfate was added[12, 14]. NPs with an average diameter of 108.1 ± 9.9 nm and a zeta potential of 27.4 ± 1.6 mV were obtained. Our results show that light-activatable NPs rapidly (minutes range) release retinoic acid (RA) when exposed to a blue laser/UV light. These NPs reduce more efficiently the clonogenicity of bone marrow tumor cells from patients with acute myeloid leukemia (AML) and induce more efficiently the differentiation of RA-low sensitive leukemia cells than NPs without light activation. Further, we show that leukemia cells transfected with light-activatable NPs containing RA can engraft into the *in vivo* bone marrow, in the proximity of other leukemic cells, be differentiated after blue laser activation, and release paracrine factors that modulate leukemic cells in the vicinity.

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