Uncovering tumor-host molecular and cellular interactions involved in tumor dormancy using polymer therapeutics

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ABSTRACT SUMMARY

Tumor dormancy has important implications for early detection and treatment of cancer. Lack of experimental models and limited clinical accessibility constitute major obstacles to the molecular characterization of dormant tumors. We have developed a model in which human osteosarcoma tumors remain dormant for a prolonged period of time until they switch to a fast-growing angiogenic phenotype. We have further developed a novel targeted combined anti-angiogenic polymer therapeutic, HPMA copolymer Alendronate-TNP-470 (HPMA-ALN-TNP-470), that successfully reverts the fast-growing tumors to a dormant phenotype. Following our hypothesis that dormant tumors undergo a stable genetic reprogramming during their switch to the fast-growing phenotype, we performed a microRNA (miR) array in order to get insights into the molecular mechanisms underlying the angiogenic switch. Based on the expression signature distinguishing dormant versus switched fast-growing tumors, we intend to identify novel early cancer and angiogenesis key regulators which could provide a rationale for development of dormancy-promoting tumor therapy strategies.

INTRODUCTION

Dormant tumors are defined as microscopic encapsulated tumors that remain asymptomatic, do not grow in mass and do not send metastases over prolonged periods of time. Although the tumor dormancy phenomenon has important implications for early detection and treatment of cancer, its biology and genetic characteristics are poorly understood (1). One of the proposed mechanisms for the escape of tumors from dormancy is the induction of angiogenesis. Failure of a tumor to recruit new vasculature results in a non-angiogenic and non-progressing dormant tumor (2). To elucidate the molecular mechanisms underlying this “angiogenic switch”, we established a pair of cell lines that generate dormant avascular and fast-growing angiogenic osteosarcomas in SCID mice. When inoculated into mice, the dormant cell line (“Saos-2-D”) remains avascular and cannot grow beyond 1 mm³ until it escapes and forms angiogenic tumors at a later stage, about a year following the initial inoculation in the mouse. The cell line isolated from the escaped tumors (“Saos-2-E”) produce large vascularized tumors within one month of their subsequent inoculation into mice.

We have recently demonstrated that treatment of a fast-growing angiogenic osteosarcoma-bearing mouse with our novel anti-angiogenic HPMA-ALN-TNP-470 (3) conjugate induced tumor dormancy for a long period of time. We hypothesized that, by exploring the molecular mechanisms which led to this prolonged dormancy period, we can shed light on the mechanisms which govern the angiogenic switch. We therefore performed a miR array of fast-growing angiogenic Saos-2-E and dormant Saos-2-D human osteosarcoma cells, either treated with our conjugate or untreated. The molecular signature distinguishing dormant versus switched fast-growing tumors was analyzed. Revealing new molecular targets involved in the “angiogenic switch” phenomenon could provide important tools for dormancy-dependent tumor therapy strategies.

EXPERIMENTAL METHODS

Our dormancy model was validated by inoculation of Saos-2 cells into immunocompromised mice. Three weeks following inoculation size-matched tumors (1-3 mm³) generated from Saos-2-D or Saos-2-E were resected and stained for the presence of proliferating and apoptotic cells and for microvessels. This pair of cells was comprehensively characterized in vitro by evaluating cell proliferation, migration and invasion. Next, mice bearing Saos-2 fast-growing tumors were treated intravenously with HPMA-ALN-TNP-470 conjugate (30 mg/kg q.o.d.), and followed-up for changes in the dormancy period. Finally, miR array of cells generating dormant or fast-growing osteosarcomas, either treated with the conjugate or untreated, was performed. miRs which signature is changed from a fast-growing to a dormant
phenotype by treatment with our conjugate were selected for further evaluation. Those miRs were overexpressed in Saos-2-E cells, and phenotypic changes were monitored.

RESULTS AND DISCUSSION

We have successfully established a tumor dormancy model in which small non-angiogenic tumors spontaneously escape from dormancy and form large angiogenic tumors. Histological staining of size-matched microscopic tumors (1-3 mm³), revealed that tumors generated from Saos-2-E are more vascularized compared to tumors generated from Saos-2-D cells. We found that despite these great differences shown in vivo, Saos-2-D and Saos-2-E cells share similar characteristics in vitro.

Systemic administration of HPMA-ALN-TNP-470 conjugate was not toxic, as opposed to the combination of the free drugs, and was able to prolong the dormancy period of Saos-2-E tumors by extending the animal’s survival by over two months (Fig. 1).

To analyze the molecular mechanisms which led to this prolonged dormancy period, miR microarray was performed on Saos-2-D and Saos-2-E cells, either treated with our conjugate or non-treated. This array pointed out several miRs as potential regulators of tumor dormancy (Fig. 2). Out of those miRs, miR-200c and miR-93, (representing either a mild or a substantial difference in the expression profile respectively), were selected for further evaluation.

miR-200c or miR-93, which were upregulated in Saos-2-D cells, were ectopically overexpressed in Saos-2-E cells. miR200c and miR93 were able to delay the angiogenic switch of Saos-2-E tumors by 60 and 90 days respectively (Fig. 3a), a remarkable delay equivalent to 6-9 years delay in tumor progression in humans (4). The effect of these miRs on the angiogenic properties was evaluated using the aortic ring sprouting assay. Sprouting of endothelial cells in the presence of conditioned media from Saos-2-E was significantly reduced following introduction of miR-93 and miR-200c to the same extent as in the presence of conditioned media from Saos-2-D cells (Fig. 3b).

CONCLUSIONS

By exploring the molecular mechanisms by which HPMA-ALN-TNP-470 conjugate prolongs the dormancy period of Saos-2-E tumors, we uncovered novel regulators of the tumor dormancy phenomenon. Overexpression of miR-200c and miR-93 significantly prolonged dormancy period of Saos-2-E tumors and reduced their angiogenic potential. We show here that our polymer therapeutics may be used as a pharmacological tool for prediction of miRs associated with the switch from dormancy. These miRs can be potentially used as anticancer therapy when bound to a delivery system.

REFERENCES


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