Using endothelial colony-forming cells as a non-invasive tool for assessing the anti-angiogenic and anti-proliferative effect of RNA Helicase inhibition

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Background

Endothelial colony-forming cells (ECFCs) are a population of endothelial progenitor cells endowed with endothelial phenotype, clonal proliferative potential, and vasculogenic capacity *in vitro* and *in vivo*[1,2]. They are increasingly used as a non-invasive strategy to study the endothelial compartment and cancer vasculogenesis[1,2]. We previously demonstrated that ECFCs isolated from the blood of patients with Kaposi's sarcoma (KS), a lymphoangioproliferative tumor associated with infection by human herpesvirus-8 (HHV8), are HHV8-infected, show higher IL-6 production and higher proliferative and vasculogenic potential than control ECFCs, suggesting that ECFCs may be putative precursors of spindle cells, which are the typical KS tumor cells[3-4]. As such, ECFCs obtained from KS patients may also represent a valuable tool for screening drug activity for KS treatment. Dead-Box ATPase dependent RNA helicase 3 (DDX3) is a multifunctional protein involved in all aspects of RNA metabolism, and a new class of antiviral and antitumoral compounds targeting DDX3 is under development.

Aim of the study

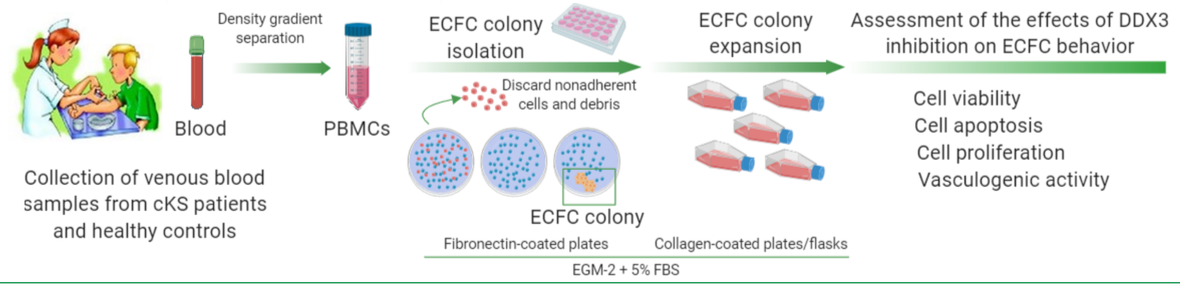
In order to investigate the efficacy of DDX3 inhibition on ECFC proliferation and vasculogenic activity, in this study we assessed the effects of a DDX3-inhibitor (DDX3-inh) on ECFCs obtained from cKS patients.

Study design and methods

5 cKS patients and 5 sex- and age-matched healthy controls were enrolled in the study (Table 1). ECFCs were isolated and cultured from peripheral blood samples by using a protocol previously optimized in our laboratory[4-5]. ECFCs isolated from both cKS patients and healthy donors were treated with DDX3-inh - a DDX3 inhibitor developed by First Health Pharmaceuticals - for 24, 48 and 72 hours. At the end of the incubation periods, ECFCs were analyzed for the following parameters: cell viability, assessed by MTT assay; cell apoptosis, assessed by Annexin-V staining; cell proliferation, assessed by crystal violet assay; vasculogenic activity, assessed by the Matrigel assay. Wilcoxon signed-rank test was used to assess the effects of treatment within each group. Mann-Whitney U-test was used for comparison between cKS patients and healthy donors.

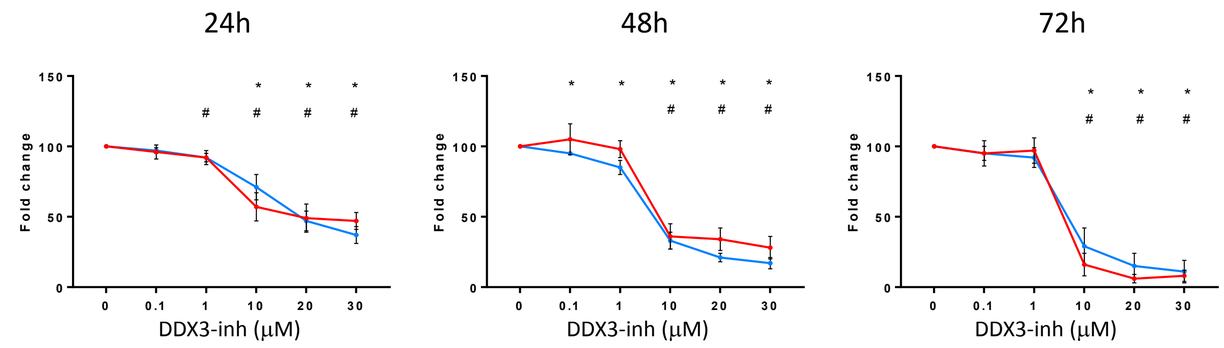
	Controls	cKS	
Number of subjects	5	5	
Age (years; average±ES; range)	73±2 (66-78)	74±2 (66-79)	P=n.s.
Sex (ratio female:male)	1:4	1:4	P=n.s.

Table1. Clinical characteristics of healthy controls and cKS patients.



Results

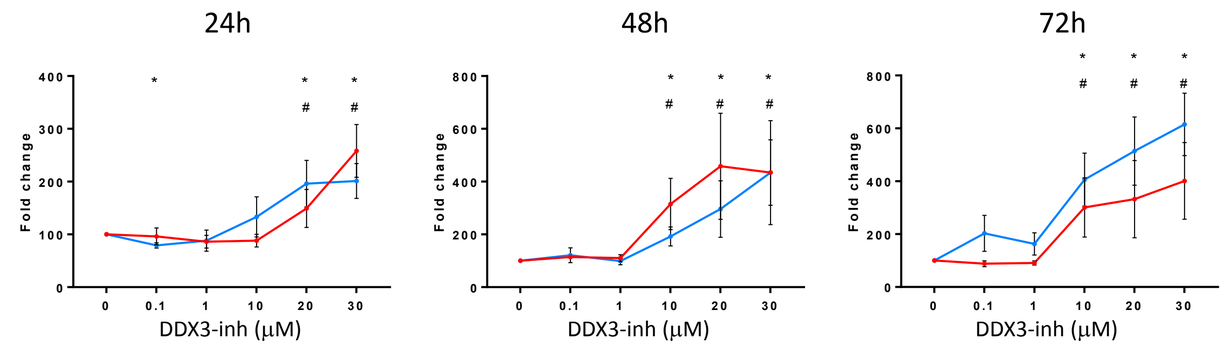
1. Cell Viability



Assessment of ECFC viability in response to DDX3 inhibition. Control ECFCs (light blue line, n=5) and cKS ECFCs (red line, n=5) were incubated for 24, 48 or 72 hours in EGM-2 medium, supplemented with DDX3-inh. Cell viability was assessed by MTT assay. Data were expressed as fold change of the absorbance normalized on untreated samples. Data shown as mean ± SEM. *P < 0.05 treated control ECFCs vs untreated control ECFCs, #P<0.05 as treated cKS ECFCs vs untreated cKS ECFCs. Statistical significance was calculated using the Mann-Whitney U test and Wilcoxon signed-rank test.

Incubation of ECFCs with DDX3-inh induced a significant reduction of cell viability, in both cKS patients and healthy donors.

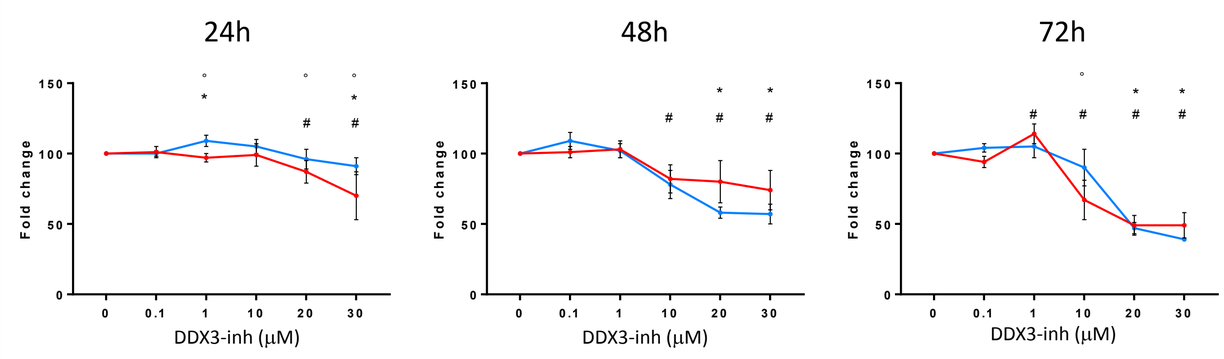
2. Cell Apoptosis



Assessment of ECFC apoptosis in response to DDX3 inhibition. Control ECFCs (light blue line, n=5) and cKS ECFCs (red line, n=5) were incubated for 24, 48 or 72 hours in EGM-2 medium, supplemented with DDX3-inh. Apoptosis was assessed by flow cytometry, and apoptotic cells were identified as Annexin-V$^+$ cells. Data were expressed as fold change of frequency of apoptotic cells normalized on untreated samples. Data shown as mean ± SEM. *P < 0.05 treated control ECFCs vs untreated control ECFCs, #P<0.05 as treated cKS ECFCs vs untreated cKS ECFCs. Statistical significance was calculated using the Mann-Whitney U test and Wilcoxon signed-rank test.

Incubation of ECFCs with DDX3-inh induced an increase of cell apoptosis, in both cKS patients and healthy donors.

3. Cell proliferation

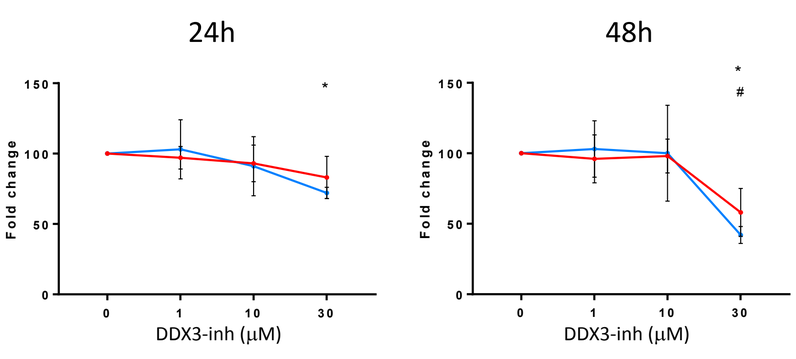


Assessment of ECFC proliferation in response to DDX3 inhibition. Control ECFCs (light blue line, n=5) and cKS ECFCs (red line, n=5) were incubated for 24, 48 or 72 hours in EGM-2 medium, supplemented with DDX3-inh. Cell proliferation was assessed by Crystal Violet assay. Data were expressed as fold change of the absorbance normalized on untreated samples. Data shown as mean ± SEM. *P < 0.05 treated control ECFCs vs untreated control ECFCs, #P<0.05 as treated cKS ECFCs vs untreated cKS ECFCs, °P<0.05 control ECFCs vs cKS ECFCs. Statistical significance was calculated using the Mann-Whitney U test and Wilcoxon signed-rank test.

Incubation of ECFCs with DDX3-inh induced a significant reduction of cell proliferation, in both cKS patients and healthy donors;

ECFCs obtained from cKS patients showed a higher sensitivity to the anti-proliferative action of DDX3-inh at the earliest time point.

4. Vasculogenic activity



Assessment of ECFC vasculogenic activity in response to DDX3 inhibition. Control ECFCs (light blue line, n=5) and cKS ECFCs (red line, n=5) were incubated for 24 or 48 hours in EGM-2 medium, supplemented with DDX3-inh. Vasculogenic activity was assessed by Matrigel assay, evaluating the number of capillary-like structures/microscopic field. Data were expressed as fold change of the number of capillary-like structures/microscopic field normalized on untreated samples. Data shown as mean ± SEM. *P < 0.05 treated control ECFCs vs untreated control ECFCs, #P<0.05 as treated cKS ECFCs vs untreated cKS ECFCs. Statistical significance was calculated using the Mann-Whitney U test and Wilcoxon signed-rank test.

Incubation of ECFCs with DDX3-inh induced a significant reduction of vasculogenic activity, in both cKS patients and healthy donors.

Conclusions

DDX3-inh was efficient in promoting ECFC apoptosis and reducing cell viability, proliferation and vasculogenic activity of ECFCs. All these effects were dose-dependent, and observed at any time point. In addition, ECFCs obtained from cKS patients showed a higher sensitivity to the anti-proliferatyive action of DDX3-inh at the earliest time point. All together, these results provide new evidence of a potential anti-angiogenic effect of DDX3 inhibition, thus paving the way for further investigations and developments of DDX3-inhibitors in the control of tumor-associated angiogenesis.

References

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