Highly sensitive detection of *IDH1* R132H mutations in plasma of glioma patients

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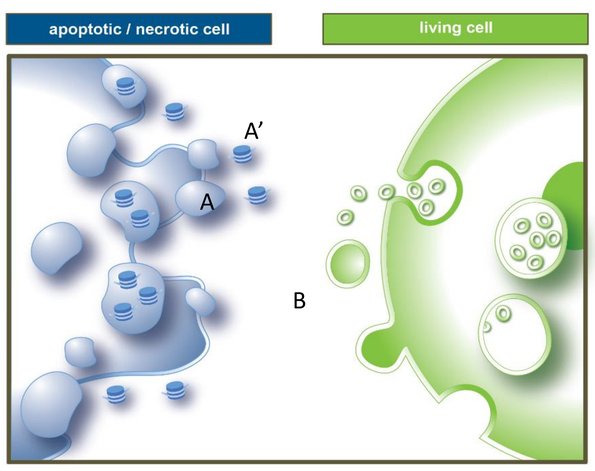
Introduction

Gliomas are the most common primary malignant brain tumors in adults. Recurrent isocitrate dehydrogenase (IDH) gene mutations are found in up to 80% of low grade gliomas and 20% of secondary glioblastomas. Cell-free DNA is released into biofluids from dying cellular processes and has been used to detect mutations in plasma from cancer patients but has been a challenge in gliomas. Blood brain barrier (BBB) is a dynamic but complex barrier that exosomes but not larger particles can cross.

On the other hand, exosomes and other extracellular vesicles are actively released from living processes into plasma, and combining these two sources of nucleic acids increases sensitivity [1,2,3]. The goal of this study was to develop a droplet digital PCR -based assay to detect the *IDH1* R132H mutation from plasma samples of glioma patients using cfDNA and exosomal nucleic acids (exoNA) combined as input material **(Figure 1)**.

Figure 1. Origin of nucleic acids in biofluids. Apoptotic or necrotic cells may release cell-free DNA (cfDNA) in apoptotic vesicles (A) or as circulating nucleosomes (A'). Exosomes are actively released by living cells (B) carrying nucleic acids into circulation (exoNA).



[2]*Adapted from Castellanos-Rizaldos et al. 2018*

Material and Methods

We developed a plasma-based assay that combines exoNA and cell free DNA to detect single copies of R132H mutation with ~2 mL of input material using ddPCR (Figure 2).

The clinical performance was assessed on an interim cohort of 16 positive and 8 negative R132H clinical samples confirmed by matched tissue data (Table 1). Three additional healthy samples were also processed as negative controls.

Figure 2

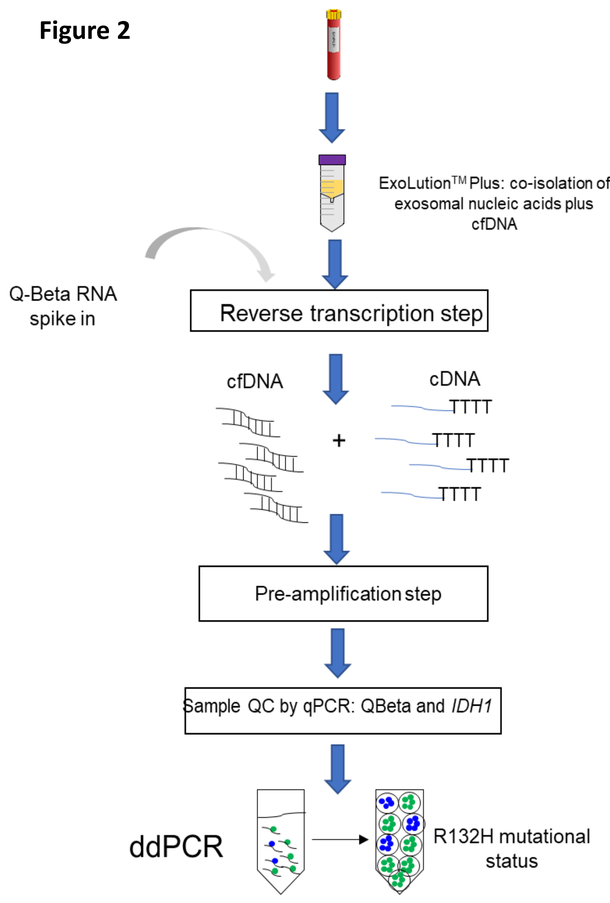


Figure 2. Assay workflow. (A) Exosome-derived nucleic acids and cfDNA isolation from plasma. (B) Reverse transcription step that includes QBeta as a control of inhibition. (C) Pre-amplification step. (D) Quality Control step by qPCR to ensure absence of enzymatic inhibitors. (E) Droplet Digital PCR that detects R132H within *IDH1*.

Table 1

Name	Age	Gender	IDH1/2 Status	IDH1/2 Status Determined by
BC-17002	65	Female	Negative	rhPCR
BC-17005	65	Male		rhPCR
BC-17007	27	Female		rhPCR;NGS
BC-17022	71	Male		rhPCR
BC-17018	65	Male		IHC
BC-17025	68	Female		rhPCR
BC-17027	63	Female		rhPCR
BC-17028	73	Female		IHC;rhPCR
BC-17004	52	Male	Positive	rhPCR
BC-17012	54	Female		IHC;rhPCR
BC-17015	52	Female		rhPCR;NGS
BC-17020	35	Male		rhPCR
BC-17011	49	Male		IHC
BC-17023	27	Male		NGS
BC-17030	47	Male		IHC
GBM15	54	Female		IHC
GBM16	28	Female		IHC
GBM23	49	Male		IHC
GBM33	48	Male		IHC
GBM35	34	Female		IHC
GBM38	33	Male		IHC
GBM40	30	Male		IHC
GBM47	27	Male		IHC
GBM50	24	Female		IHC

Results

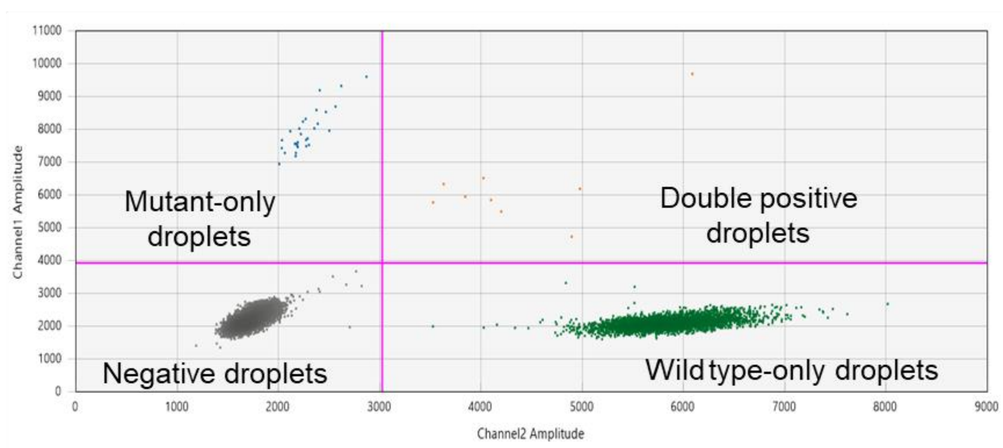
We detected R132H in the patient samples analyzed with 56% sensitivity and 91% specificity (Figure 3, Table 2). This result is similar to the sensitivity we previously published using exosomes from cerebrospinal fluid[4], however plasma is a much more accessible biofluid for diagnostics.

Table 2. Confusion matrix that summarizes the clinical samples tested.

		Plasma results using cfDNA and exosomal NA		Total number
		+	-	
Tissue result*	+	9	7	16
	-	1	10	11

*All four healthy samples have been included as tissue negative.

Figure 3. Example of droplet digital PCR results from one of the R132H positive patient samples by tissue. 2-Dimensional plot that shows a clear separation between the different clusters of droplets (positive for either channel or negative).



Conclusions

- **First novel platform able to capture both exoNA and cfDNA from plasma from patients with low grade gliomas to detect R132H.**
- **Comparable sensitivity to a previous study that used Cerebrospinal Fluid, with the advantage of being less invasive.**
- **This is a promising result for brain cancer since this is a particularly challenging disease for peripheral biomarkers.**
- **When the same exoNA isolation platform was utilized across 307 patients in different peripheral cancers this platform achieved a sensitivity ranging from 90-98% and specificity of 92%.**

References

1. Möhrmann Let al. Clinical Cancer Research, 2017.
2. Castellanos-Rizaldos et al. Clinical Cancer Research, 2018.
3. Krug AK et al. Annals of Oncology, 2017.
4. Chen et al, Molecular Therapy Nucleic Acids 2013.

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