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Letter to the Editor

MiRNA-155 and colorectal cancer, the role of real time PCR in laboratory diagnosis

Dear Editor,

We were very interested to read the article published by Liu et al[1] regarding the involvement of microRNA-155 (miR155) on the invasion ability of colon-rectal cancer (CRC) cells. The authors have designed an *in vitro* approach with SW-480 cell lines that proves an active role of miR-155 in β-catenin overexpression. This mechanism should be connected with the cancer cell invasion rate[1]. CRC is a modern-day health problem in developed countries and is the third most commonly diagnosed tumor and the fourth leading cause of cancer death in the world. Its early clinical diagnosis is based on colonoscopy after a positive fecal occult blood test[2-5]. The subsequent laboratory diagnosis considers histological-histochemical exams and molecular procedures in order to discover the mutations of CRC oncogenes such as BRAF[6]. Although this analytical approach has resulted as being extremely useful for a precise and sensitive diagnosis of CRC in loco, it has often proved to be incapable of forecasting the invasion attitude of an initial neoplastic lesion, with consequent uncertainty in the disease prognosis[2]. The linkage between miR-155, β-catenin, and cancer has been evaluated for different tumors. β-catenin is a subunit of a protein complex acting as a signal transducer (Wnt signaling) that has a role in different cell functions, for example, embryogenesis. Its overexpression is associated with many types of cancers, e.g., breast, lung, and CRC. One cause of this mechanism is an increase in the cellular cytoplasm of miR-155. This is a single-stranded non-coding RNA (ncRNA) which contains approximately 19-25 nt developed from hairpin precursor molecules containing 70-120 nt (Figure 1). As described by Liu et al[1], upregulating the expression of miR-155 in CRC SW-40 cells promotes the distant cell invasion and consequently the tumor metastasis. This preliminary work could be interesting in the CRC diagnosis and understanding of cancer pathogenesis, in particular, we have focalized three main points: (a) it can provide ideas for new protocols in the CRC personalized treatment, for example by blocking the expression of miR-155 and the tumoral activity of Wnt/β-catenin system; (b) it suggest a clinically validate laboratory assay for a precise quantification of the miR-155 level in tissue for tumor detection factor, but also as a signal of prognosis outcome. Amongst currently available techniques for profiling miR-155, described by Liu et al[1], as well as other authors[7] (Table I), the most frequently used is the quantitative Real-time PCR (qRT-PCR).

Available qRT-PCR Procedures for miR-155 Expression

This procedure generally shows a good dynamic range of quantitation and also has a low cost and good sensitivity. But the molecular fluorescent probe, used for target detection, represents a crucial point in this procedure and as shown in Table I, three typologies of molecular probes: SYBR Green I, TaqMan Probe and Molecular Beacon, are used with miR-155 experiments. These probes have already been studied in different experiments for RNA/DNA targets in Eukaryota/Virus, Bacteria[8-14], whose proprieties are extensively known.

SYBR Green

The SYBR family have derived cyanine dyes used currently in molecular biology for the non-specific staining of nucleic acids. SYBR Green I is able to bind DNA and the resulting double-stranded DNA-dye complex shows a max absorption at λmax = 497 nm, and emits λmax = 520 nm in green light. As shown in Table I, this methodology has been used to detect miR-155 in a

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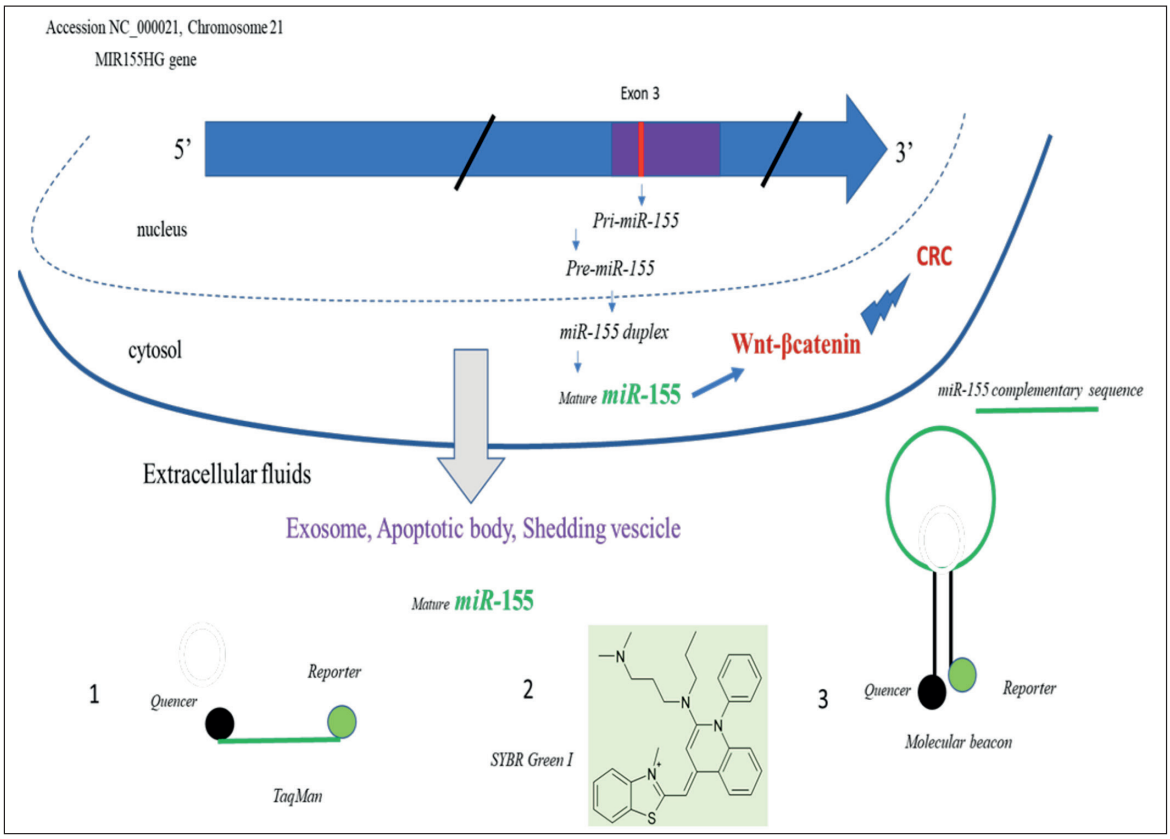

Figure 1. miR-155 processing and available qRT-PCR probes used for its detection.

variety of samples from serum to stool-tissue. The advantage of SYBR Green I is that no probes are required, and consequently, the assay setup and running costs can be reduced when the PCR primer is well designed[9,10]. Its main disadvantages are the presence of false-positive signals in the PCR reaction, due to the presence of non-specific double-stranded DNA sequences such

Table I. Overview on molecular methods used for miR-155 detection[7].

Probe/procedure	Method	Sample	Cancer	Studies
TaqMan	Real time PCR	Plasma, serum, sputum, tissue, urine	AML, breast, lung, pancreas, thyroid	9
SYBR Green I	Real time PCR	Plasma, serum, tissue, stool	B cell lymphoma, ESCC, lung, NPC, OSCC, Pancreas.	14
Molecular Beacon	Real time PCR-confocal microscopy	Tissue	Lung.	3
Microarray-probe	Microarray	Bone marrow, serum, tissue	AML, breast, CML, colon, CRC, endometrium, GC, glioma, HC, kidney, lymphoma, LP, lung, melanoma, OC, OV, prostate.	33
Deep sequencing equencing	Next generation fluid, tissue	Cells pancreatic	HC, Lung, Pancreas	3

Legend: AML = acute myeloid leukemia, CML = cronic myeloid leukemia, CRC = colon-rectal cancer, ESCC = esophageal squamous-cell carcinoma, AML = acute myeloid leukemia, HC = hepatocellular carcinoma, GC = gastric cancer, LP = liposarcoma, OSCC = oral squamous cell carcinomas, NPC= nasopharyngeal carcinoma, OV = ovarian cancer.

as primer dimer products. This is why a melting curve analysis can be performed. These assays are normally designed to detect mature MiRNA and generally showed a sensitivity of 0.05-0.08 ng RNA/PCR with different DNA targets[9,11].

TaqMan Probe

TaqMan represents a hydrolysis fluorescent probe that uses the 5' nuclease activity of the Taq DNA polymerase, which allows molecular methods to be used only to detect specific amplification products. The probe contains a fluorescent reporter dye on the 5' end and a quencher dye on the 3' end and, thus, the proximity of the quencher dye greatly reduces the fluorescence caused by the reporter dye[8]. If the miR-155 target is present, the probe anneals downstream from one of the primer sites and is cleaved by the 5' nuclease activity of Taq DNA polymerase as this primer is extended. As a consequence, this reaction separates the reporter dye from the quencher, due to the increase in the reporter dye signal. In comparison with SYBR Green I, the advantage of this procedure lies in a specific hybridization between probe and target (TaqMan RBG).

Molecular Beacons

The Molecular Beacon MB is a hairpin-shaped oligonucleotide probe, which has the property of fluorescing upon hybridization with a specific target sequence[6]. Its hairpin shape increases target specificity providing a lower background signal compared to that found in other linear probes. MB is an extremely specific probe in comparison with SYBR Green and TaqMan[8,13,15]. The applications for miR-155 expression profile with this probe are different and regard qRT-PCR base methods, but it can also be used for in situ detection in tissue fragments with confocal microscopy using MB combined with chitosan.

Conclusions

Liu et al[1] have suggested the use of miR-155 as a novel candidate for the diagnosis of tumor metastasis. These experimental data have demonstrated that this molecular target could be useful to predict tumor typology and progression in the early stages of the disease. By comparing this data with the PCR base methods published for miR-155 expression, a variety of procedures are proposed with different sensitivity and specificity ranges, which means that it is essential to standardize different procedures and probes.

Conflict of Interest
The Authors declare that they have no conflict of interests.

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