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Extracellular RNA in urine may provide useful biomarkers for muscular dystrophy

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Massachusetts General Hospital (MGH) researchers have found that extracellular RNA (exRNA) in urine may be a source of biomarkers for the two most common forms of muscular dystrophy, noninvasively providing information about whether therapeutic drugs are having the desired effects on a molecular level. The report published in online journal *Nature Communications*, is the first to show that urine exRNA can be used to monitor systemic diseases that do not directly affect the urinary tract.

"Our findings could facilitate drug development by offering a convenient, painless and relatively low-cost assay that may reduce and perhaps eventually eliminate the need for multiple invasive muscle biopsies to track disease activity and therapeutic response," says Thurman Wheeler, MD, MGH Department of Neurology, senior author of the report. "Urine exRNA monitoring could determine whether a drug is reaching its target long before clinical effects on muscle function could be detected and may enable early identification of whether dosage adjustments may be required, something that would be impossible with invasive muscle biopsies."

There are several types of muscular dystrophy, all of which lead to progressive muscle weakness and loss of muscle mass. Duchenne muscular dystrophy (DMD) is the most common form, with symptoms that usually begin in children under the age of 5. Myotonic muscular dystrophy (DM) is the most common adult-onset form and has two subtypes—DM1 and DM2. Each form is caused by a different gene

mutation. In DMD, the mutation affects the gene for dystrophin, a protein essential to the strength of muscle fibers. DM-associated mutations—in the DMPK gene for DM1 and in the CNBP gene for DM2—involve excessive repeats of nucleotides, leading to abnormal processing of RNA molecules.

The mutation associated with DM1 affects RNA splicing, the process that removes non-coding segments from an RNA molecule. A single gene can normally give rise to several different proteins, with the differences being determined by alternative RNA splicing patterns. The DM1 mutation interferes with appropriate splicing of RNAs encoding several other proteins, and analysis of RNA splice variants in muscle biopsies has been used to determine disease severity in patients. In animal models, splice variants in muscle tissue have been used to indicate whether potential therapies are reaching their molecular target. A less invasive way of assessing disease severity and therapeutic response could expand the number of patients who could receive therapeutic drugs or participate in clinical trials. For example, a recent clinical trial for a DM1 drug was restricted to adult patients partially because of the need for repeat muscle biopsies.

Carried through bodily fluids like blood and urine in membrane bubbles called vesicles, exRNA encompasses messenger, non-coding, and microRNA molecules and can reflect mutations, deletions and other molecular variants. A few muscular-dystrophy-associated RNA or protein biomarkers have been identified in the blood of patients. Even though it seemed unlikely that exRNA from the skeletal and cardiac muscle tissues affected by DM1 could pass through the kidney's filtration system into the urine, urine is such an easily accessible fluid that Wheeler's team analyzed vesicles from both blood and urine for exRNAs that could reflect results of the DM1 mutation.

Their experiments comparing urine exRNAs from DM1 patients,

patients with two other forms of muscular dystrophy and unaffected control volunteers identified 10 transcripts that are alternatively spliced in a pattern unique to DM1 patients, most of which had been previously found in patient muscle biopsies. A composite biomarker incorporating these 10 transcripts was 100 percent accurate in distinguishing DM1 patients from unaffected controls in a different group of participants. Samples taken from untreated DM1 patients over several months indicated consistency of splicing patterns within an individual, suggesting that repeat sampling could accurately reflect disease state and treatment response.

Along with developing a more precise assay for rapid measurement of alternative RNA splicing in urine and other bodily fluids, the team showed that splicing patterns in total RNA from the cells in the urine were different from and less useful as biomarkers than those from exRNAs and found that exRNAs in blood could not distinguish between DM1 patients and controls. They also found that the splicing patterns of some urine exRNA transcripts reflected the severity of DM1 symptoms, and that a small group of asymptomatic patients with the DM1 mutation had urine exRNA splicing patterns midway between those of symptomatic patients and unaffected controls.

A group of drugs being evaluated for the treatment of DMD manipulate splicing of the dystrophin gene in order to remove a specific exon—a protein-coding segment of RNA—producing a shortened but partially functional version of the dystrophin protein. The MGH team showed that urine exRNAs from six untreated DMD patients accurately reflected the specific gene mutation in each patient. In two DMD patients being treated with eteplirsen—an FDA-approved DMD drug that induces skipping of the target exon—analysis of urine exRNA was able to confirm the drug was reaching its molecular target, the first such confirmation not provided by muscle biopsy.

"Our demonstration of disease-specific splice variants in urine exRNA suggests the value of biofluids as a means of identifying novel splice variants that may help correlate gene variants with symptoms for several diseases for which noninvasive biomarkers are unavailable," says Wheeler, an assistant professor of Neurology at Harvard Medical School. "These findings support studies of exRNA from urine, blood or cerebrospinal fluid as biomarker replacements for tissue biopsies in other conditions with altered RNA splicing—including other types of muscular dystrophy, spinal muscular atrophy and amyotrophic lateral sclerosis."

More information: Layal Antoury et al, Analysis of extracellular mRNA in human urine reveals splice variant biomarkers of muscular dystrophies, *Nature Communications* (2018). DOI: 10.1038/s41467-018-06206-0

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