Micro-RNA Levels Higher in Aggressive Melanomas

BY SHARON WORCESTER
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CHICAGO — Micro-RNA-21 and micro-RNA-155 are expressed at significantly higher levels in primary malignant melanoma tumor samples than in samples from benign nevi—and in indeterminate melanocytic lesions with an aggressive histological phenotype, according to data presented at the American Society of Clinical Oncology annual meeting.

Samples from eight dermal nevi, 28 malignant melanomas, and 49 pathologically indeterminate melanocytic lesions were evaluated using real-time polymerase chain reaction. Investigators found a mean 7.6-fold increase in micro-RNA-21 expression ($P = .0001$) and a mean 13.3-fold increase ($P = .0001$) in micro-RNA-155 expression, compared with the benign nevus samples, reported Gregory B. Lesinski, Ph.D., of Ohio State University, Columbus.

Another potential biomarker—micro-RNA-21b—showed a trend toward increased expression, but the difference did not reach statistical significance in this small sample size ($P = .07$). However, micro-RNA-21b remains of interest, Dr. Lesinski noted.

Micro-RNA-21 and micro-RNA-155 also appear to be expressed at higher levels in certain indeterminate melanocytic lesions. Indeterminate lesions, including deep penetrating nevi, dysplastic nevi, and Spitz nevi, pose a particular challenge in that diagnosis and determination of the appropriate course of action are uncertain.

A false-positive diagnosis could lead to unnecessary treatment; a false-negative diagnosis could lead to undertreatment and increased risk of melanoma development.

In this study, expression of micro-RNA-21 and micro-RNA-155 was analyzed in indeterminate lesions with more than one mitosis per 10x high-power field.

When they were compared with those with less than one mitosis per 10x high-power field, there was significantly higher expression of micro-RNA-21 ($P = .0005$) and micro-RNA-155 ($P = .04$).

'Micro-RNA-21, at least, may in fact be a relevant marker that can be used to complement the traditional histological analyses used to diagnose these lesions.'

Melanocytic lesions that were greater than 1 mm in depth, compared with thinner lesions, also had significantly higher levels of micro-RNA-155 ($P = .01$), suggesting these might have more malignant potential; micro-RNA-21 was higher in the thicker lesions as well, but the difference did not reach statistical significance, Dr. Lesinski said.

In a subset of 13 patients with indeterminate lesions whose lesions were considered suspicious enough that the patients were sent for sentinel node biopsy, micro-RNA-21 and micro-RNA-155 expression were compared with expression in benign nevi—in both those that proved node positive and those that proved node negative.

Micro-RNA expression was significantly higher in the lesions from node-positive patients, compared with expression in benign nevi (mean 6.5-fold increase), than in node-negative patients, compared with expression in benign nevi (1.3-fold increase).

"Surprisingly, we didn't find the same relationship for micro-RNA-155; the data were highly variable [for micro-RNA-155] regardless of whether the patients were node negative or node positive," said Dr. Lesinski, who reported he has no disclosures relevant to the data he presented.

These data are "very preliminary," he added, noting that the sample size for this portion of the study is being expanded to include at least 50 patients to make a more accurate statistical determination about the expression of these micro-RNAs.

"Nonetheless, these data are very enticing to us, and they suggest that micro-RNA-21, at least, may in fact be a relevant marker that can be used to complement the traditional histological analyses used to diagnose these lesions," he said.

The expression of micro-RNAs—a group of more than 200 recently identified noncoding molecules considered to be a new class of oncogenes—is altered in many types of tumors.

This study is among the first to explore this expression in melanoma. Studies to further assess the potential value of micro-RNAs as diagnostic and prognostic tools in melanoma are ongoing, Dr. Lesinski said.

During a discussion of Dr. Lesinski's findings and those from other melanoma biomarker studies, Elizabeth Grimm, Ph.D., said they are part of a "fabulous set of new data."

Dr. Lesinski's findings regarding indeterminate lesions are particularly important given the therapeutic dilemma they pose, she said.

She expressed concern, however, about difficulty in determining whether micro-RNA expression is increased in melanomas, compared with indeterminate lesions. Differences were marked between melanoma and benign nevi, but based on her review of the data, it appears there was little difference in expression between melanoma and indeterminate lesions, Dr. Grimm said, adding that research regarding other micro-RNAs should continue.

She also noted that she is curious about whether there is a micro-RNA signature that adds value to the current staging system and markers commonly used, and whether micro-RNAs can be used independently of common diagnostic markers used by pathologists, such as MAGE, MART, and S100B.

While many questions remain, she applauded the research effort, noting that there are valid markers in other tumor types; thus, the "noble" efforts to identify molecular markers for melanoma are worthwhile. "We're not just dreaming—we will have them for melanoma some day," said Dr. Grimm of M.D. Anderson Cancer Center, Houston. ■

Gene Loss May Explain Gender Differences in Melanoma

BY SHARON WORCESTER
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CHICAGO — A specific pattern of X and Y chromosome losses—and in particular the loss of an important tumor-suppression gene—was shown in a recent study to be associated with melanoma progression.

This "very new and very unexpected finding" could help explain gender differences that are seen in melanoma, such as the increased risk of death in men with melanoma, compared with women with melanoma, Dr. Alan Spatz said at the American Society of Clinical Oncology annual meeting, where he presented the findings.

'PPP2R3B appears from this study to be a very important tumor suppression gene whose loss is associated with tumor progression.'

Frozen sections from 48 melanomas—taken from 32 women and 16 men with a median follow-up of 4 years—were analyzed as part of a European Organisation for Research and Treatment of Cancer (EORTC) study. Analyses of DNA and mRNA were conducted; the end point was distant metastasis-free survival.

A significant correlation was found between DNA copy number and mRNA level for 1,455 genes (P less than .05). In the women's samples, losses in the X chromosome were significantly associated with distant metastasis-free survival ($P = .009$), and the affected X chromosome was always the inactive X.

In the men, losses in the Y chromosome were significantly associated with distant metastasis-free survival ($P = .015$), reported Dr. Spatz of Institut Gustave Roussy in Villejuif, France.

Further investigation to identify any particular gene or genes that fulfilled the criteria of being located on the X chromosome in women and escaping inactivation of X chromosome, as well as being located on the Y chromosome in men, showed that only the PPP2R3B gene, also know as PR48, did so.

The normalized expression of the gene, which is located on Xp22 in women and on Yp11 in men was found to be associated with distant metastasis-free survival at a "totally unexpected level of significance," ($P = .0007$), after adjusting for clinical and pathologic prognostic variables, including gender, age, ulceration, and thickness, Dr. Spatz said.

PR48 is a lesser-known subunit of a well-known and very important gene (serine/threonine protein phosphatase 2A) which he described as "kind of a gatekeeper in cell biology," he said.

PR48 mediates the dephosphorylation of CDC6 by phosphatase 2A and controls initiation of DNA replication in human cancer cells, especially melanoma, he explained.

The findings, along with those from other ongoing studies that are designed to help improve understanding of PPP2R3B biology, could potentially provide biologic clues regarding gender differences in melanoma progression and survival; the gender effect in melanoma is strong—men have been shown to have a relative excess risk of death of 1.87, compared with women with melanoma.

"We can conclude that there is a very specific pattern of X and Y chromosome losses associated with melanoma progression … and that PPP2R3B appears from this study to be a very important tumor suppression gene whose loss is associated with tumor progression.

"Does this explain the gender effect? Maybe if indeed we show that there is a differential frequency of inactivated X chromosome in females, as compared with Y chromosome—but this is still an open question," Dr. Spatz said.

In a discussion of the findings, Elizabeth Grimm, Ph.D., a professor at the University of Texas M.D. Anderson Cancer Center, Houston, called these and other emerging data a "snapshot of finest biomarker work in melanoma at this time." Dr. Spatz's work is particularly provocative, she said.

She asked, however, how—given the findings—one would explain that the incidence of melanoma in young women is higher than that in young men. The increased incidence in men only begins to become evident around age 50 years, she noted, questioning whether other lifestyle parameters are in play.

"Just food for thought," she said.

Dr. Grimm also noted that it would be interesting, should the findings be validated in the ongoing studies that Dr. Spatz mentioned, to see how they might change the course of treatment or provide prognostic information, and to determine whether some form of gene therapy to redeliver the X and Y chromosome losses could be developed. ■