PAR-4 tees off lung cancer

By Michael J. Haas, Senior Writer

A paper in the *EMBO Journal* presents the first *in vivo* mechanistic evidence showing how a deficiency in prostate apoptosis response-4 protein contributes to Ras-induced non-small cell lung cancer. And it identifies the protein and a molecule downstream of it—protein kinase C-ζ—as potential new targets to treat the indication.[1] Companies contacted by *SciBX* agreed that the paper reveals a novel role for PAR-4 as a tumor suppressor, but said that translating the findings into a viable therapeutic strategy would be difficult.

The research team included scientists from the **University of Cincinnati College of Medicine**, the **Spanish National Cancer Research Institute**, **The Biotechnology Centre of Oslo** and the **Hospital Universitario Puerta de Hierro** in Madrid. The team leaders, both from the University of Cincinnati, were Maria Díaz-Meco, associate professor of cancer and cell biology, and Jorge Moscat, chairman of cancer and cell biology.

A key feature of the study was the development of an animal model deficient in prostate apoptosis response-4 (PAR-4; PAWR) protein. "There have been many *in vitro* studies that identify PAR-4 as a tumor suppressor *in vitro*, but none *in vivo*," Díaz-Meco told *SciBX*. "Until a PAR-4 knockout model was generated, the *in vivo* mechanisms could not be elucidated."

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PAWR-less mice

PAR-4 is most highly expressed in normal prostate and endometrial cells. *In vitro* studies have focused on the role of PAR-4 deficiency in spontaneously arising prostate and endometrial cancers.[2–4] For the *EMBO J* study, the researchers wanted to know how PAR-4 deficiency contributes to *in vivo* tumorigenesis caused by genetic mutations.

They decided to look at PAR-4 deficiency in Ras-induced lung cancer for two reasons: PAR-4 is also highly expressed in normal lung tissue; and at least 25% of all human lung adenocarcinomas—the most common non-small cell lung cancer (NSCLC) tumor type—result from a mutation in the signal transducer Ras, which has been linked to PAR-4 deficiency.[5,6]

To investigate the potential link between PAR-4 and NSCLC, the team first looked for correlations between loss of PAR-4 expression and different types of tumors in human lung tissue. They found a strong correlation—47%—between PAR-4 loss and adenocarcinomas.

When the adenocarcinomas were stratified by tumor grade, the team found an even stronger correlation between PAR-4 loss and malignancy—59% for grade I–II tumors and 74% for grade III tumors. By comparison, only 6% of squamous cell carcinomas showed PAR-4 loss. These results supported the team's hypothesis that loss of PAR-4 played a role in tumorigenesis in NSCLC.

Next the team developed a new animal model by breeding *PAR-4* knockout mice with Ras oncogene–bearing mice. The PAR-4–deficient offspring had a higher incidence of lung tumors, and shorter lifespans, than Ras oncogene–bearing mice with wild-type *PAR-4* gene—confirming that PAR-4 deficiency contributed to Ras-induced tumorigenesis.

But the team also found that levels of PAR-4 expression in mice that did have the gene were constant, regardless of whether the mice had the mutated *Ras* or not. This suggested that, in contrast to what had been suggested in some earlier *in vitro* studies, Ras does not downregulate PAR-4 *in vivo*.

This was surprising, according to Díaz-Meco because "in 1999 we published evidence that Ras can downregulate PAR-4 *in vitro* and in xenografts, and that reconstituting PAR-4 reduced tumor size."[7]

The team then analyzed tumors from the PAR-4 knockout mice and found that both NF-κB and protein kinase B (PKB; Akt) were activated. This indicated that PAR-4 could suppress two tumorigenic pathways, one involving NF-κB—which matched the team's previous findings in PAR4-deficient prostate and uterine cells[8,9]—and a novel pathway involving activation of Akt, which contributes to tumor growth and survival. The team confirmed the latter finding by silencing PAR-4 expression in human cells with short interfering RNA.

Lastly, the team investigated the mechanism by which PAR-4 deficiency results in Akt activation.

Previous studies by Díaz-Meco, Moscat and other groups showed that PAR-4 inhibited the activity of protein kinase C-ζ (PKC-ζ), both *in vitro* and *in vivo*.[2–5,10] This led the current Díaz-Meco–Moscat team to postulate that PKC-ζ mediates Akt activation in lung cells. Indeed, *in vitro* studies demonstrated that PKC-ζ phosphorylated Akt at residue Ser124, a step that is critical to subsequent phosphorylations that fully activate Akt.

Collectively, the team's findings show that loss of PAR-4's tumor-suppressing effects contribute substantially to tumorigenesis in Ras-induced lung cancer, and indicate that PAR-4 and PKC-ζ are potential targets for treating NSCLC.

"The paper unveils a new and completely unexpected role for PAR-4 in Akt activation," Díaz-Meco said. "It places PAR-4 among other tumor suppressors known to regulate Akt, such as PTEN. The more we study PAR-4, the more similar to PTEN it appears to be."

PTEN (phosphatase and tensin homolog deleted on chromosome 10; MMAC1; TEP1) encodes a proapoptotic protein that is found in nearly all tissues in the body and blocks Akt activation. It is one of the most commonly lost or mutated tumor suppressors involved in cancer.

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>
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Unprecedented suppressor

Jerry McMahon, chairman and CEO of **Poniard Pharmaceuticals Inc.**, said that the findings of the Díaz-Meco–Moscat team demonstrate a tissue-specific role for PAR-4 that is novel for a tumor suppressor.

"Their claim about the tumor-suppressing function of PAR-4 is different from other well known tumor suppressors which act on the cell cycle," he said.

As an example, McMahon cited p53, which is found in all normal tissue types and exerts a proapoptotic action on cells in response to DNA damage. Elimination or mutation of the p53 gene potentiates a malignant phenotype in any tissue type, he noted. By contrast, PAR-4 has elevated expression in certain normal tissues—lungs, prostate and endometrium—such that its absence or mutation enables tumorigenesis specifically in those tissues.

"This is unprecedented—that expression in normal tissue dictates how important a target might be in cancer," said McMahon.

"Another aspect of the tissue tropism in this work is that PAR-4 is more important in Ras-induced adenocarcinomas," he said. "The researchers are making a case for PAR-4 loss being skewed towards adenocarcinomas and greater malignancy."

McMahon said the team's hypotheses about PAR-4 and its tumor-suppressing actions were logical and indisputable. Nonetheless, he said it would be desirable to see the tissue-specific role of PAR-4 confirmed by other researchers.

Poniard has picoplatin, an intravenous platinum-based chemotherapy, in three clinical trials: a Phase III to treat small cell lung cancer in patients who have relapsed or are refractory to other platinum-based chemotherapies; a Phase II trial as a first-line treatment for colorectal cancer; and a Phase II trial to treat hormone-refractory prostate cancer.

The company also has an oral formulation of picoplatin in a Phase I trial.

Difficult PAR-4

Though companies contacted by *SciBX* did not dispute the novelty of the findings in the *EMBO J* paper, they differed over which strategy—reconstitution of PAR-4 function, inhibition of PKC-ζ, or neither—was likely to be effective in treating NSCLC. They did agree that developing therapeutic agents for either proactive strategy would be challenging.

McMahon said that restoring PAR-4 function in tumors would be the better strategy, because of PAR-4's specific role in lung cancer. "The authors make a more compelling case for PAR-4 than for PKC-ζ as a target in lung adenocarcinomas," he said.

But McMahon acknowledged it would be more difficult to restore PAR-4 expression than to inhibit PKC-ζ. "Gene therapy and reconstitution haven't worked for other tumor suppressors," he noted. "p53 especially has been the focus of much effort in this regard."

Likewise, he said, reconstitution of PAR-4 function in a tumor cell having a deleted or mutated PAR-4 gene might be difficult. But "investigations in that direction could lead to some other mechanism which would allow reconstitution of what PAR-4 does, if not of the protein itself."

"PKC-ζ might be more tractable in terms of developing an inhibitor for it, but it is less of a target because one can imagine it has other functions besides phosphorylating Akt," McMahon said. He noted that most serine/threonine kinases—which include PKC-ζ—have multiple cellular functions.

He said another question is whether targeting PKC-ζ would sufficiently mimic, and therefore compensate, for the loss of, PAR-4—a strategy that would depend on how specifically PAR-4 acts on the kinase.

"Tumor suppressors typically interact with many other molecules," McMahon noted. "Maybe in cancer all PAR-4 does is downregulate PKC-ζ—maybe not." If PAR-4's action is indeed that specific in cancer, PKC-ζ would be a viable target, he said.

Like McMahon, Fernando Doñate, director of preclinical R&D at **Proacta Inc.**, said targeting PKC-ζ might be a more feasible strategy than restoring PAR-4—but didn't think either strategy was optimal.

"I do not see how one could target PAR-4, since one would need to replace the loss of PAR-4 with gene therapy, which is not a good approach," he said. Targeting PKC-ζ might be more feasible, "but I do not know enough to assess the possible toxicities associated with that."

Doñate acknowledged that recombinant PAR-4 might counter its deficiency, but noted that such an approach would be "quite difficult, because PAR-4 would need to be internalized, which does not work for proteins."

The usual issues associated with biologics would also come into play with recombinant PAR-4, he added, such as manufacturing cost and frequently poor pharmacokinetic profiles.

Likewise, Doñate said that targeting Akt directly would be challenging. "As far as I remember, there are issues of toxicity and specificity about which isoforms of Akt to inhibit," he said.

Doñate suggested a better approach would be targeting other upstream activators of Akt, such as phosphoinositide 3-kinase (PI3K) and mammalian target of rapamycin (mTOR; FRAP; RAFT1). "Inhibition of Akt with PI3K inhibitors is a very hot area right now," he said. As an example, he cited **Piramed Ltd.**, recently acquired by **Roche**, which is developing PI3K inhibitors to treat cancer.

Consequently, Doñate said, "I do not see much commercial application of the *EMBO J* findings—unless targeting PKC-ζ directly is feasible—since the relevance of the Akt pathway to cancer was well known before."

Proacta plans to take PR-104, a hypoxia-activated, small-molecule prodrug, into two Phase II trials in combination with other chemotherapies in 2009.

Lance Leopold, CMO of **Ascenta Therapeutics Inc.**, said that PAR-4 and PKC-ζ were only potential—not validated—targets and that it was not possible to say which was better. But he noted that "current technologies favor developing inhibitors of PKC-ζ, compared to reconstituting PAR-4."

Leopold added it is not yet clear whether the two molecules play a role in cancer beyond tumorigenesis. "The PAR-4/PKC-ζ pathway may be an early event in neoplastic transformation that may not be relevant once additional transforming events occur," he said.

Although the paper "builds on the story of PAR-4 as a true tumor suppressor gene," Leopold said that the potential importance of the findings would depend on the scope and mechanism of PAR-4 inactivation in patient-derived tumor samples.

Ascenta has AT-101, a small molecule inhibitor of Bcl-2 and myeloid leukemia cell differentiation protein (Mcl-1), in a Phase I/II trial to treat hormone-refractory prostate cancer.

The back nine

Díaz-Meco agreed with all three companies that PAR-4 deficiency would be difficult to address therapeutically. "It might be easier to target the activity of the kinase [PKC-ζ], but it has been really difficult to find a specific inhibitor for any atypical PKC," she said.

PKC-ζ, PKC-λ and PKC-ι belong to one of three classes of PKC isoforms, the atypical PKCs (aPKCs). The other two classes are the conventional and novel isoforms.

Because aPKCs share about 99% homology, selective targeting of any one of them is difficult and there are no known compounds that do so, Díaz-Meco said. This presents a significant obstacle to research because the therapeutic implications of targeting an aPKC can't be examined without sorting out their specific functions, she noted.

"Our future studies will investigate whether other PKCs besides PKC-ζ mediate PAR-4 activity," Díaz-Meco said. "To that end, we are trying to identify which roles are redundant among the atypical PKCs and which are specific to each of them."

She said the team was generating PKC-λ knockout mice and seeking to identify specific inhibitors of the different aPKCs.

Another set of planned experiments will investigate how and why Ras apparently downregulates PAR-4 *in vitro* but not *in vivo*. Díaz-Meco said the team hypothesizes that the discrepancy arises from differences in Ras levels—which were higher in the previous *in vitro* studies than they were in the current studies of mice that endogenously express Ras oncogene.

"We want to develop an *in vivo* model in which Ras could be more inducible, to see if higher levels of endogenous Ras could downregulate PAR-4," she said.

Díaz-Meco added the team is writing a paper about its latest studies of PAR-4 deficiency in prostate cancer.

"The main message is that PAR-4 has synergies with other tumor suppressors in prostate cancer," she said. "Knockout of PAR-4, in cooperation with knockout of other tumor suppressors, leads to invasive prostate cancer," whereas knockout of PAR-4 alone results only in benign prostatic hyperplasia (BPH), she said.

Díaz-Meco said the findings reported in *EMBO J* are not patented.

REFERENCES

1. Joshi, J. *et al. EMBO J.*; published online July 24, 2008; doi:10.1038/emboj.2008.149
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2. Moscat, J. & Díaz-Meco, M. *Cell Cycle* **2**, 71–72 (2003)
3. Goswami, A. *et al. Mol. Cell* **20**, 33–44 (2005)
4. Moscat, J. *et al. Cell Death Differ.* **13**, 702–711 (2006)
5. Moreno-Bueno, G. *et al. Cancer Res.* **67**, 1927–1934 (2007)
6. Bos, J. *Cancer Res.* **49**, 4682–4689 (1989)
7. Barradas, M. *et al. EMBO J.* **18**, 6362–6369 (1999)
8. Garcia-Cao, I. *et al. EMBO Rep.* **4**, 307–312 (2003)
9. Garcia-Cao, I. *et al. EMBO Rep.* **6**, 577–583 (2005)
10. Díaz-Meco, M. *et al. Cell* **86**, 777–786 (1996)

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