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Perspective

Cancer Prevention Research

Adaptor Proteins as Targets for Cancer Prevention

Perspective on McCampbell et al., p. 290

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Abstract

This perspective on the report by McCampbell et al. in this issue of the journal (beginning on page 290) addresses the role of insulin receptor substrate (IRS) proteins in cancer progression. The IRS proteins link many cell-surface receptors to signal transduction pathways. Activation of the phosphoinositide-3 kinase/Akt/mammalian target of rapamycin axis normally results in serine phosphorylation and subsequent downregulation of these adaptor proteins. The authors show that changes in the negative feedback regulation of IRS proteins is associated with the progression of endometrial epithelial cells to hyperplasia and cancer. Therefore, understanding the function of adaptor proteins could provide additional strategies for cancer prevention and treatment. *Cancer Prev Res; 3(3); 263–5. ©2010 AACR.*

I recently found myself staring at an electrical wall socket that had two slots, and the plug at the end of my electrical cord had three prongs. I did not have the appropriate adaptor and, thus, was unable to connect the outside signal (electricity) to the inside system (my computer). I was stuck, unable to progress.

In an analogous fashion, cell communication with the extracellular environment requires the transmission of an outside signal to the cell interior. Once this signal is appropriately transmitted, the cell is able to do something relevant to its function. In the case of cancer cells, most signal responses are not desirable to the host. The past 20 years have seen the identification of either the targets that transmit the signal across the membrane (receptors) or their intracellular effector proteins (signal transduction molecules). Development of small molecules or antibodies that disrupt the enzymatic activity or binding sites of key effector molecules has translated into new cancer drugs. Growth factor receptors have proved to be particularly attractive targets for anticancer drug development. Many more targets are being validated in clinical trials with a myriad of drugs. These strategies have brought new attention to downstream molecules in the signal transduction pathway as potential targets. Therapeutic advances with some targets, such as estrogen receptor α, are very relevant to cancer prevention. And thus it has become clear that a better understanding of signaling pathways has the potential to improve cancer outcomes via better treatment and prevention strategies.

In most receptor systems, adaptor proteins are necessary to connect the transmembrane receptors to their appropriate signal transduction pathways. The term "adaptor protein" connotes a passive, perhaps boring, part of the signal transduction pathway. Certainly, an adaptor protein does not have any exciting enzymatic function to target, serving merely as a platform to assemble the complex signal initiated by the transmembrane receptor.

Insulin receptor substrate (IRS) proteins were among the first adaptor proteins to be identified because of their function in transmitting insulin receptor signaling. Five IRS proteins are expressed in humans, but only IRS-1 and IRS-2 have been shown to play a significant role in cancer. An excellent recent review details the known functions of the IRS proteins (1). Beyond their role in insulin and insulin-like growth factor (IGF) signaling, this family of adaptor proteins clearly has an important role in the signal transduction of many transmembrane receptors. The IRS proteins also function in the signaling of prolactin, growth hormone, anaplastic lymphoma kinase, and vascular endothelial growth factor. IRS in these settings can be tyrosine phosphorylated at multiple tyrosine sites, resulting in numerous effector proteins docking to it. Thus, IRS adaptor proteins serve to integrate the multiple potential signals transmitted by the initial receptor tyrosine kinase event.

IRS adaptor proteins have an established role in mouse models of cancer. Ma et al. (2) have shown that IRS-2 plays an important role in tumor metastasis in a model of mouse mammary carcinogenesis involving gene deletion for *IRS-1* and *IRS-2*. Animals without IRS-1 but that retained IRS-2 had enhanced mammary cancer metastasis. Using a transgenic overexpression model, Dearth et al. (3) showed that both IRS-1 and IRS-2 enhanced mammary gland hyperplasia, tumorigenesis, and metastasis. Although these differences in the role of the IRS species may be related to different types of animal models, it is clear that levels of the IRS adaptor protein affect the malignant phenotype, which is enhanced and augmented by expression of IRS-1 and/or IRS-2.

It has long been known that IRS proteins also contain multiple serine/threonine phosphorylation sites. Serine

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phosphorylation plays a critical role in IRS signal transduction, and phosphorylation at these sites can dictate IRS interactions with other signaling pathways. For example, serine-307 phosphorylation of IRS-1 is associated with decreased function of the insulin receptor. This association is known because serine-307 phosphorylation of IRS-1 results from tumor necrosis factor-α (TNF-α) activation of its own receptor and elevated TNF-α occurs in the inflammatory disease diabetes (4). IGF and insulin also downregulate their own signaling pathway through phosphorylation at specific serine IRS sites via negative feedback pathways. This negative feedback system was shown most directly in cells treated with rapamycin analogues, which inhibit the mammalian target of rapamycin (mTOR). mTOR activation results in IRS phosphorylation at serine sites, causing ubiquitination of IRS-1. Blocking this pathway with rapamycin analogues abrogates this feedback and allows continued insulin receptor signaling to phosphoinositide-3 kinase (PI3K) and Akt (5). Thus, mTOR inhibition sustains IRS expression and results in continued PI3K activation due to continued insulin/IGF-I signaling.

In this issue of the journal, McCampbell et al. (6) show that IRS phosphorylation plays a central role in the progression of normal cells to hyperplasia in human endometrium. Most endometrial hyperplasia tissue specimens expressed both IRS-1 and IRS-2, yet IRS phosphorylation on serine-636/639 was lost in hyperplasia (compared with normal cells) even in the presence of an activated PI3K/Akt/S6 kinase pathway. Therefore, the beneficial negative feedback role was functioning in normal cells but not apparently in endometrial hyperplasia and cancer.

To further explore this pathway, the authors examined female Eker rats with germline heterozygosity of the tuberous sclerosis gene 2 (*Tsc2*). The product of this interesting gene functions normally by binding to and suppressing the action of mTOR complex 1 (mTORC1). Therefore, loss of Tsc2 results in upregulation of the mTORC1 pathway, which is associated with a clinical syndrome involving multiple benign tumors. Tsc2 regulates the same rapamycin-sensitive pathway that effects IRS phosphorylation. All of the aged female rats had endometrial carcinoma. Similar to results in the human endometrial tissue specimens, loss of IRS phosphorylation on serine-636/639 was associated with, and elevated mTOR activity was maintained in, hyperplasia. Last, the authors inhibited the mTOR pathway in these animals with the rapamycin analogue WAY-129327, thus showing that blockade of mTOR activity decreased endometrial hyperplasia.

Taken together, these data suggest that the mTOR pathway is important in endometrial tumorigenesis, although development of hyperplasia also depends on the loss of negative feedback of the IRS proteins. The authors conclude that mTOR activation precedes the loss of this negative feedback because even normal tissue in Eker rats had elevated S6 kinase, a downstream target of mTOR, while continuing to show IRS-1 serine phosphorylation. Therefore, the feedback signaling pathway was intact during mTOR activation in normal cells. However, when IRS-1 serine phosphorylation was lost, the cells became hyperplastic. Interpreted another way, the loss of negative feedback of the IRS proteins is more important than mTOR activation in the transition from normal endometrium to hyperplasia. Although the exact molecular mechanism for this loss of IRS inhibition was not described, the data suggest that the adaptor proteins IRS-1 and IRS-2 (IRS-2 could not be studied directly because it lacks suitable reagents showing expression in tissues) are not just passive bystanders but could be a critical link in endometrial tumorigenesis.

Several critical questions remain, starting with, "What is the mechanism for the loss of IRS serine phosphorylation?" An obvious pathway would be the upregulation of serine/threonine phosphatases. The role of phosphatases in the insulin receptor signaling pathway is well understood. Overexpression of the dual-specificity phosphatase MKP-4/DUSP-9 enhances insulin sensitivity via its effects on IRS-1 phosphorylation (7).

What pathway or pathways are affected by the lack of IRS downregulation in endometrial cells? Obesity and higher circulating insulin levels are associated with the risk of endometrial cancer, making it plausible that these effects are mediated by insulin receptor signaling. Although insulin/IGF signaling is an attractive explanation, it must be remembered that other pathways (e.g., TNF-α) also use the IRS proteins and could have a role in endometrial cancer.

Are there functional differences between IRS-1 and IRS-2? As mentioned above, the different adaptor protein species may have a different function in malignant transformation. The potential for IRS proteins to affect both growth and metastasis is of particular interest. Therefore, loss of feedback may not only affect the transformation of the endometrial epithelium but also may influence the malignant behavior of the tumor.

Last, can interruption of protein-protein interactions be a way to prevent cancer? The work described here suggests that disrupting IRS linkage to downstream pathways could be an effective method for endometrial cancer prevention and treatment. We have been extraordinarily successful in developing small-molecule antagonists that disrupt enzymatic activity or the binding of other small molecules to proteins (mostly hormones), but less effective in developing small molecules that block protein-protein interactions. The work of McCampbell et al. (6) suggests that mTOR inhibition may not be successful for preventing endometrial cancer because it would result in persistence of IRS-1 and/or IRS-2. Perhaps an effective "one-two" punch would be to downregulate mTOR and disrupt IRS protein interactions to optimally target molecular events in the malignant transformation of endometrial cells.

Just as in the absence of a needed electrical wall-socket adaptor, nothing much happens in signal transduction without the appropriate adaptor in place. McCampbell

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et al. show that the IRS proteins are one of the important adaptors for signal transduction in endometrial carcinogenesis, with a critical role in the development of hyperplasia and cancer. In this case, losing the adaptor could have important implications for cancer prevention and treatment.

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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