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Letter to the Editor

Impact of Epithelial Organization on Myc Expression and Activity—Response

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We agree with Partanen and colleagues that both their original published work (1) and ours (2) show that epithelial architecture suppresses c-Myc and that the fundamental point of disagreement between our studies relates to the mechanism through which this occurs. Klefstrom argues that this phenomenon arises due to processes that attenuate the biologic response to Myc; our data indicate that epithelial architecture acts, in large part, by suppressing *c-Myc* gene transcription. Because discriminating between these two possibilities is crucial to understanding how solid tumors arise, it is important to know whether cellular architecture is a barrier to Myc function *per se* or whether Myc expression accounts for the observations that have been made in this area.

Three lines of evidence support our conclusion that changes to *Myc* gene expression, not Myc protein activity, are responsible for the reported influence of epithelial cell architecture on c-Myc. First, unlike Partanen and colleagues (1), we compared exogenous c-Myc (and MycER) expression side-by-side in 2- versus 3-dimensional (3D) cultures and showed directly that both endogenous and exogenous Myc expression drop significantly as acini mature (ref. 2, Figs. 1 and 3). Although Partanen and colleagues suggest that this may be due to the method we used to harvest proteins for immunoblotting, we note that reviewers of our articles were supplied with a figure showing that extended trypsinization of cells in 2D cultures, identical to that conducted with 3D cultures, does not reduce exogenous c-Myc (or MycER) levels beyond what is observed from direct scraping and lysis of the cells. Thus, trypsinization *per se* does not appear to effect a global reduction in Myc protein levels as suggested. Second, we note that the changes in exogenous Myc protein levels we reported are mirrored at the level of Myc transgene mRNA and that transgene mRNA levels drop rapidly in 3D cultures, being about 5-fold lower at day 8 than day 0 (ref. 2, Fig. 3B and C). As RNA was harvested by direct guanidinium lysis of cells in 3D culture, without trypsinization, any concerns dealing with sample preparation and handling are moot. These data provide compelling evidence that Myc transgene expression is downregulated in 3D cultures, making it impossible to directly compare the effects of Myc expression by this method in 2D versus 3D settings. Finally, we reported that adenoviral expression of Myc in 3D cultures, which we confirmed by quantitative immunoblotting and reverse-transcriptase real-time PCR analysis drives Myc expression at levels almost identical to those seen by retrovirus-mediated gene transfer in 2D cultures, potently stimulates apoptosis in this setting. Thus, if Myc is expressed at identical levels in 2D and 3D cultures, its apoptotic functions are preserved. We certainly cannot exclude an influence of architecture on Myc function based on our studies, but our finding that a significant decrease in Myc transgene expression (at the level of mRNA and protein) occurs in organized epithelial structures and the demonstration that expressing Myc at equivalent levels in 2D and 3D settings potently induces apoptosis make this unlikely.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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