Plakoglobin is a cytoplasmic protein and a homologue of β-catenin and Armadillo of *Drosophila* with similar adhesive and signaling functions. These proteins interact with cadherins to mediate cell-cell adhesion and associate with transcription factors to induce changes in the expression of genes involved in cell fate determination and proliferation. Unlike the relatively well-characterized role of β-catenin in cell proliferation via activation of c-MYC and cyclin D1 gene expression, the signaling function of plakoglobin in regulation of cell growth is undefined. Here, we show that high levels of plakoglobin expression in plakoglobin-deficient human SCC9 cells leads to uncontrolled growth and foci formation. Concurrent with the change in growth characteristics we observe a pronounced inhibition of apoptosis. This correlates with an induction of expression of BCL-2, a prototypic member of apoptosis-regulating proteins. The BCL-2 expression coincides with decreased proteolytic processing and activation of caspase-3, an executor of programmed cell death. Our data suggest that the growth regulatory function of plakoglobin is independent of its role in mediating cell-cell adhesion. These observations clearly implicate plakoglobin in pathways regulating cell growth and provide initial evidence of its role as a pivotal molecular link between pathways regulating cell adherence and cell death.

Plakoglobin (γ-catenin) is a structural and functional homologue of β-catenin and Armadillo (Arm, the product of the *Drosophila* segment polarity gene) with dual adhesive and signaling roles (1, 2). Structurally, plakoglobin and β-catenin proteins consist of a central core of 12–13 arm repeats flanked by the N- and C-terminal domains. Arm repeats are protein interaction domains functioning as binding/docking sites for protein partners that in many cases have been shown to interact in an overlapping and mutually exclusive manner (3–5). The central core repeats of plakoglobin and β-catenin function as binding sites for various cellular proteins including cadherins, the transcription factors of LEF/TCF family, and the tumor suppressor protein adenomatous polyposis coli (APC) (1, 6). The N-terminal domain regulates the stability of plakoglobin and β-catenin proteins, whereas the C-terminal domain has an apparent transactivation ability (1, 7). Plakoglobin and β-catenin interactions with cadherins are necessary for the maintenance of intercellular adhesion and proper functioning of epithelia (8). β-Catenin regulates the interactions between the classical cadherins and the cytoskeleton at adherens junctions, whereas plakoglobin interacts with both classical and desmosomal cadherins. Its interaction with the desmosomal cadherins is essential for desmosome assembly, whereas plakoglobin-classical cadherin complexes appear to have a regulatory function (1, 9, 10). In addition, β-catenin and plakoglobin function as the terminal components of the Wnt signaling pathway regulating cell fate determination and differentiation during development (4). Furthermore, high levels of cytosolic β-catenin can participate in signaling pathways regulating cell proliferation (11). The mechanism of β-catenin function in regulation of cell proliferation is well characterized in carcinoma cells and occurs via its interactions with LEF/TCF transcription factors. The cytosolic β-catenin interacts with LEF/TCF, and β-catenin-LEF-TCF complex is translocated into the nucleus where it activates the expression of genes involved in cell proliferation (12–17). On the other hand, the potential role of plakoglobin in regulation of cell proliferation is not determined although this protein appears to subscribe to all functions performed by β-catenin.

The maintenance of cell number in normal tissue homeostasis and in development is achieved by regulated balance between cell proliferation and programmed cell death or apoptosis. The key regulators of the apoptotic process, the BCL-2 protein family, includes both pro-survival and pro-apoptotic members (for recent reviews see Refs. 18 and 19). The expression of the BCL-2 family is regulated, primarily, by cytokines although some members are induced as part of the p53-mediated damage response (Ref. 20 and references therein). Pro-survival proteins such as BCL-2 have been suggested to inhibit apoptosis by blocking the function of caspases, cytochrome c, and a host of other proteins implicated in the execution of programmed cell death in organisms from nematodes to humans (reviewed in Ref. 21). Here we present evidence that shows that high levels of plakoglobin can induce unregulated growth concurrent with induction of expression of the anti-apoptotic protein BCL-2 and the inhibition of caspase-3 activation and apoptosis. In addition, this growth regulatory function of plakoglobin appears to be independent of its role in mediating cell-cell adhesion.

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§ Contributed equally to this work.

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†† Medical Scientist of the Alberta Heritage Foundation for Medical Research, Medical Research Council of Canada Distinguished Scientist, and a Howard Hughes Scholar.

§§ To whom correspondence should be addressed: 6–24 Medical Sciences Bldg., Dept. of Cell Biology, University of Alberta, Edmonton, Alberta T6G 2H7, Canada. Tel.: 780-492-3356; Fax: 780-492-0450; E-mail: mpasdar@ualberta.ca.

The abbreviations used are: LEF/TCF, lymphoid enhancer factor/T-cell factor; APC, adenomatous polyposis coli; TUNEL, terminal transferase-catalyzed dUTP end labeling; PIPES, 1,4-piperazinediethanesulfonic acid; CMV, cytomegalovirus.
**EXPERIMENTAL PROCEDURES**

**Cells and Culture Conditions**—The human squamous carcinoma cells SCC9 have been derived from a tongue carcinoma (American Type Culture Collection (ATCC)) and do not express plakoglobin (22). SCC9 cells were maintained in minimum essential medium (Sigma) supplemented with 10% fetal bovine serum.

When appropriate, staurosporine was added to the media at 500 nM, and cultures were washed for various lengths of time from 1 to 5 h. Cells were then processed for either immunofluorescence or Western blotting using the TUNEL reagent or anti-caspase antibodies, respectively.

**Plasmid Construction and Transfection**—The mammalian expression plasmid pREP-Pg expressing low levels of plakoglobin has been described (22). The plasmid expressing high levels of plakoglobin was constructed by cloning the full-length plakoglobin cDNA (22) into the mammalian expression plasmid pBK-CMV at the NheI site, adjacent to the CMV promoter. The construct was designated pBK-CMV-Pg. Transfections were performed with 25 μg of either pBK-CMV or pBK-CMV-Pg and LipofectAMINE reagent (Life Technologies, Inc.) according to the manufacturer’s protocol. Forty eight hours post-transfection, medium was replaced with minimum essential medium containing 400 μg/ml G418. G418-resistant colonies were selected and screened for plakoglobin expression using immunofluorescence and immunoblot assays. Positive colonies expressing plakoglobin were subcloned by limiting dilution to obtain single-cell isolated colonies.

**Cell Growth and Aggregation Assays**—For growth measurement, SCC9 and low and high plakoglobin-expressing SCC9 cells were each plated at 2 × 10^5 cells in replicate 35-mm culture dishes. At 3, 5, 7, 10, 14, and 18 days after plating, cultures were trypsinized and counted.

Aggregation assays were performed as described (23) and entailed triplicate confluent 60-mm culture dishes of SCC9 and low and high plakoglobin-expressing SCC9 cells as stocks for single cell suspensions. Following trypsination (0.04% trypsin in phosphate-buffered saline containing 1 mM CaCl2) of each culture, replicates of 2 × 10^5 single cells were transferred to 2-ml aliquots. Cells were incubated at 37 °C with constant shaking at 90 rpm for a total of 2 h. At 30-min intervals 200-μl aliquots were withdrawn, and the number of single cells were counted using a hemocytometer. The percentage of aggregation represents the percent decrease in the number of single cells at each time point.

**Western and Northern Blotting**—Total cell extracts were prepared by direct solubilization of control or staurosporine-treated cultures in SDS sample buffer. Proteins were separated by SDS-polyacrylamide gel electrophoresis and processed for immunoblotting with anti-plakoglobin (24), anti-α- and β-catenin (Sigma), anti-BCL-2 (Upstate Biotechnology, Inc.), or anti-caspase-3 (kindly provided by D. Nicholson, Merck Frosst, Quebec, Canada) antibodies and developed using ECL. For Northern blot analysis, total RNA from vector-transfected and low and high plakoglobin-expressing SCC9 cells as well as BCL-2-transfected Jurkat cells was extracted and probed with a 32P-labeled human BCL-2 cDNA probe. Equal amounts of total protein (250 μg) or RNA (25 μg) were loaded in each lane.

**Cell Fractionation and Immunoprecipitation**—Cells were extracted in the 100-mm culture dish with 1.5 ml of cytoskeleton extraction (CSK) buffer (300 mM sucrose, 10 mM PIPES, pH 6.8, 50 mM NaCl, 3 mM MgCl2, 0.5% (v/v) Triton X-100, 1.2 mM phenylmethylsulfonyl fluoride, 0.1 mg/ml DNase, and 0.1 mg/ml RNase (22)). This extract buffer allows the separation of the cytoskeleton-associated (insoluble) pool of cadherins and catenins from the cytoplasmic (soluble) pool of these proteins. Cells were removed from the dishes with a rubber policeman and centrifuged at 48,000 × g for 10 min. The soluble fraction was removed from the insoluble pellet. The pellet was resuspended in 150 μl of CSK buffer (1% SDS, 10 mM Tris-HCl, pH 7.5, 2 mM EDTA, 0.5 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride) and boiled for 10 min. Prior to immunoprecipitation, the SDS concentration was reduced to 0.1% by the addition of CSK buffer. The soluble and insoluble fractions were precleared for 30 min by incubation with 10 μl of preimmune sera and 35 μl of Pansorbin cells (Calbiochem) as described (22). After a 7-min centrifugation at 14,000 × g, precleared extracts were divided into culture aliquots of 250 μg of total protein and processed for immunoprecipitation with 3–5 μl of either plakoglobin or a mixture of antibodies against N-cadherin (Sigma) and desmosomal catenins (22) and 35 μl of protein A-Sepharose CL-4B (Amersham Pharmacia Biotech) beads as described (22). To ensure complete depletion, each aliquot was immunoprecipitated three consecutive times.

**Immunofluorescence Microscopy**—For plakoglobin staining, cells were fixed with formaldehyde and extracted with cytoskeleton extraction buffer as described previously (22). For nuclear labeling, cells were grown on coverslips and remained untreated or were incubated in 500 nM staurosporine from 1 to 5 h. Coverslips were air-dried, fixed with freshly prepared 4% paraformaldehyde in phosphate-buffered saline for 30 min, permeabilized for 2 min on ice with 0.1% Triton X-100 in 0.1% sodium citrate, and blocked for 30 min with 20% fetal bovine serum and 3% bovine serum albumin. Nuclei were then labeled with either TUNEL reagent (Roche Molecular Biochemicals) according to the manufacturer’s protocol or Hoechst 33342 (1 μg ml−1; Sigma) for 15 min.

**RESULTS**

**High Levels of Plakoglobin Expression Induces Unregulated Growth and Foci Formation**—The mammalian expression plasmids pREP-9-Pg and pBK-CMV-Pg (see “Experimental Procedures”) were used, respectively, to express wild type human plakoglobin at low and high levels in the plakoglobin-deficient SCC9 cells. The parental SCC9 cell line and the low plakoglobin-expressing transfectants have been characterized previously (22, 23). Total cell extracts from 4 independent clones of the low plakoglobin-expressing (Fig. 1A, top) and high plakoglobin-expressing transfectants (Fig. 1A, bottom) were analyzed for plakoglobin expression by immunoblotting. Quantitation of the immunoblots showed that there was, at least, 20-fold more plakoglobin protein in the high plakoglobin-expressing transfectants (data not shown).

SCC9 cells have the typical fibroblastoid morphology (Fig. 1B, a) and growth properties of transformed cells. Expression of plakoglobin at low levels induced epidermoid morphology (Fig. 1B, b; also see Ref. 22). In contrast, when plakoglobin was expressed at higher levels, SCC9 cells began to grow on top of each other and formed numerous foci (Fig. 1B, c and d). This observation was also confirmed by the growth properties of the different transfectants and untransfected/vector-transfected SCC9 cells. Growth analysis of parental SCC9 cells showed that at single cell density, these cells grew slowly until they became subconfluent (~5 days) and then the rate of growth increased and remained high for up to 14 days. After 2 weeks in culture, SCC9 cells began to die (Fig. 1C, solid circles, also see Ref. 22). In contrast, the low plakoglobin-expressing transfectants exhibited contact inhibition of growth and began to die upon reaching confluency (within 5–7 days; Fig. 1C, open circles, also see Ref. 22). SCC9 cells expressing high levels of plakoglobin continued to grow long after attaining confluency and formed numerous foci (Fig. 1C, open diamonds). We monitored the relative amount and subcellular distribution of plakoglobin in low and high plakoglobin-expressing transfectants (Fig. 1D). In the low expressers, plakoglobin is primarily localized to the areas of cell-cell contact in a punctate distribution typical of the desmosomal junctions (Fig. 1D, b). In the high-expressing transfectants, however, plakoglobin is distributed homogeneously throughout the cell without any distinct peripheral localization (Fig. 1D, c), a pattern also observed in the foci throughout the culture (Fig. 1D, d).

**Inhibition of Apoptosis in High Plakoglobin-expressing SCC9 Transfectants**—Programmed cell death regulates growth and morphogenesis in epithelial cells and is, in turn, regulated by both cell-cell and cell-matrix adhesion (25–28). To determine whether the uncontrolled growth in the high plakoglobin-expressing transfectants was mediated by the inhibition of apoptosis, we examined the occurrence of staurosporine-induced apoptosis in different transfectants (Fig. 2). Duplicate cultures of low- and high plakoglobin-expressing transfectants remained untreated (controls) or were incubated in 500 nM staurosporine for 5 h. Cultures were then processed for detection of apoptotic nuclei by immunofluorescence staining using terminal transferase-catalyzed dUTP end labeling (TUNEL, Fig. 2, a–d) or stained with Hoechst 33342 (Fig. 2, e–h). As shown in Fig. 2, fragmented, apoptotic nuclei were undetectable in con-
controls and the high plakoglobin-expressing transfectants (Fig. 2, a–c and e–h). In contrast, both TUNEL and nuclei staining revealed the presence of numerous apoptotic nuclei in the low plakoglobin-expressing cells (Fig. 2, d and h).

**Induction of BCL-2 Expression in High Plakoglobin-expressing SCC9 Transfectants**—The anti-apoptotic protein BCL-2 has been shown to inhibit apoptosis induced by a variety of cytotoxic insults including staurosporine treatment (for a review see Ref. 20). To determine whether inhibition of apoptosis in the high plakoglobin-expressing transfectants was mediated by BCL-2, total cell extracts from both the low and high plakoglobin-expressing transfectants were processed for immunoblotting with an anti-BCL-2 antibody. Fig. 3A shows immunoblots of total cell extracts from typical low (top) and high
plakoglobin-expressing transfectants. We did not detect any BCL-2 protein in the low plakoglobin-expressers, whereas the high plakoglobin-expressing cells showed easily detectable levels of this protein. This observation was confirmed by Northern blot analysis in which total RNA isolated from untransfected/vector-transfected and plakoglobin-transfected SCC9 cells hybridized with anti-BCL-2 antibodies. SCC9 cells were used as a positive control. The position of the 18 S and 28 S RNAs are indicated. The lower band in BCL-2 transfected Jurkat cells may represent a shorter transcript (42).

**Fig. 3. Plakoglobin-overexpressing SCC9 transfectants show increased expression of BCL-2.** A, immunoblot analysis of total cell extracts from low and high plakoglobin (Pg)-expressing SCC9 cells hybridized with anti-BCL-2 antibodies. B, Northern blot analysis of total RNA isolated from vector-transfected and plakoglobin-transfected SCC9 cells. BCL-2-transfected Jurkat cells were used as a positive control. The position of the 18 S and 28 S RNAs are indicated. The lower band in BCL-2 transfected Jurkat cells may represent a shorter transcript (42).

**Inhibition of Caspase-3 Activation in High Plakoglobin-expressing SCC9 Transfectants**—Caspases are cysteine proteases that have been implicated in the execution of programmed cell death (21). Caspases are synthesized and maintained in an inactive proenzyme form and are proteolytically activated upon the appropriate signals (21). Genetic and biochemical evidence suggests that pro-survival proteins such as BCL-2 and inhibition of the caspase cascade. Disruption of cadherin-mediated adhesion has been suggested to contribute to the unregulated growth during tumorogenesis (31). To this end we examined the adhesive properties of the high plakoglobin-expressing transfectants. Fig. 5A shows the aggregation ability of the plakoglobin-deficient SCC9 cells (Fig. 5A, solid circles) and low (Fig. 5A, open circles) and high (Fig. 5A, open diamonds) plakoglobin-expressing transfectants. Aggregation assays measured the decrease in the number of single cells as described under “Experimental Procedures” and led to the following observations. In SCC9 cells, we detected ~35% decrease in the number of single cells within the first 30 min followed by a small increase in the number of aggregates within the next 90 min; while by the end of the assay time, ~45% of SCC9 cells were present in aggregates (Fig. 5A, solid circles, also see Ref. 22). In low plakoglobin-expressing transfectants, at 30 min, there were approximately twice as many aggregates as those detected in SCC9 cultures (68 versus 35%). Within the next 60 min, there was a further increase (to 77%) that remained unchanged for the rest of the assay period (Fig. 5A, open circles, also see Ref. 22). For the high plakoglobin-expressing transfectants we observed ~55% decrease in the number of single cells within the first 30 min followed by a rapid increase in the number of aggregates within the next 30 min (~70%) and a further slight increase (~73%) by the end of 120-min assay time (Fig. 5A, open diamonds). Therefore, the cell proliferative ability of the excess plakoglobin appears to be independent of its contribution to the increased adhesive properties of the SCC9 cells. Further support for this observation was obtained by characterizing the soluble (cytoplasmic), insoluble (membrane-associated), and the cadherin-independent pools of plakoglobin in the low and high plakoglobin-expressing cell lines (Fig. 5B).

For the experiments performed in Fig. 5B, cultures were grown to confluency and extracted with CSK buffer to separate the soluble from the insoluble fractions. Following cell fractionation, aliquots of 250 μg of total protein from each fraction of each cell line were processed for immunoprecipitation with plakoglobin or cadherin antibodies as described under “Experimental Procedures.” The amount of the soluble and insoluble plakoglobin in each cell line was determined by separation of the plakoglobin-containing immune complexes on SDS 6% polyacrylamide gels followed by immunoblotting with the anti-plakoglobin antibodies (Fig. 5B, top). The amount of the cadherin-independent plakoglobin in each fraction for each cell
line was detected by Western blotting of the cadherin-depleted supernatants from the cadherin immunoprecipitates using plakoglobin antibodies (Fig. 5B, bottom). As shown in Fig. 5B, top, in the low plakoglobin-expressing cells, all of the cellular plakoglobin was detected in the insoluble pool. In contrast, in the high plakoglobin-expressing cells, plakoglobin was not only detected in both pools, its level was significantly higher in the soluble fraction. Examination of the cadherin-depleted soluble and insoluble extracts from both lines detected plakoglobin in the cadherin-depleted soluble extract from the high plakoglobin-expressing cells exclusively (Fig. 5B, bottom). Together, these observations suggest that it is the untethered and cadherin-independent plakoglobin that likely mediates the effects observed in the high plakoglobin-expressing cells.

**Decreased β-Catenin Levels in Plakoglobin-expressing SCC9 Cells**—Characterizing the cadherin-catenin complexes in SCC9 cells and the low plakoglobin-expressing transfectants, we previously showed that plakoglobin expression led to a reduction in the endogenous β-catenin levels (22). Fig. 6 is an immunoblot of an equal amount of total cell lysates from SCC9 cells and the low and high plakoglobin-expressing transfectants. It shows that the amount of decrease in β-catenin levels corresponds to the levels of the exogenously expressed plakoglobin. Quantitation of the immunoblot showed a reduction of ~40 and 80% in the β-catenin levels in the low and high plakoglobin-expressing cells, respectively (data not shown).

**DISCUSSION**

Plakoglobin is a multifunctional cytoplasmic protein that interacts with a number of cellular partners involved in pathways regulating cell adhesion and cell proliferation. Binding to the cadherins directs plakoglobin into the adhesion pathway, whereas binding to the tumor suppressor protein APC marks the protein for degradation. Similar to β-catenin, the N-terminal domain of plakoglobin includes consensus serine and threonine phosphorylation sites for glycogen synthase kinase-3β. Phosphorylation of these residues is necessary for degradation of the soluble cadherin-independent plakoglobin or β-catenin (1). Degradation requires interaction with APC and phosphorylation of both proteins by glycogen synthase kinase-3β. This is facilitated by axin which interacts with all three proteins simultaneously (32–34). The phosphorylated APC/plakoglobin (or β-catenin) is then rapidly degraded via the ubiquitin-proteosome pathway (35, 36). If not sequestered by cadherins and...
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APC, the excess plakoglobin or β-catenin will be available to interact with other binding partners. The excess TCF/LEF-associated β-catenin activates genes involved in cell proliferation (15-17). Interestingly, it was recently shown that this complex can also activate the expression of genes regulating neuronal apoptosis during retinal development in Drosophila embryos with mutations in D-APC (37). Similarly, humans with germ line APC mutations, and presumably high levels of cytoplasmic β-catenin, exhibit increased frequency of apoptotic cells in colon polyps (38). In contrast to the relatively well characterized role of β-catenin in regulation of cell growth, the growth regulatory function of plakoglobin has not been investigated.

To gain further insights into the signaling function of plakoglobin, we overexpressed plakoglobin in plakoglobin-deficient SCC9 cells (22, 23). Our previous studies had shown that at low levels plakoglobin expression was able to induce contact inhibition of growth and a fibroblast to epidermoid transition associated with the formation of desmosomes and increased adhesivity (22). The present study shows that when expressed at high levels, however, plakoglobin induced unregulated growth and foci formation. Examination of the adhesive properties of the low and high plakoglobin-expressing transfectants showed similar increases in their aggregation abilities relative to the parental SCC9 cells. Complexes of plakoglobin-N-cadherin and desmosomal cadherins were detected in all transfectants; however, a large cadherin-independent pool of plakoglobin was only detected in the high plakoglobin transfectants (Fig. 5B). Since the high plakoglobin-expressing cells demonstrate enhanced growth, this suggests that the signaling role of plakoglobin in regulation of cell proliferation is mediated via the cadherin-independent pool of plakoglobin and is independent of its adhesion function. It is noteworthy that plakoglobin expression in SCC9 cells led to a corresponding reduction in the levels of the endogenous β-catenin (Fig. 6; also see Refs. 22 and 37). Complexes of β-catenin-N-cadherin were detected in both the low and high plakoglobin-expressing transfectants despite the reduced levels of β-catenin in these transfectants (Ref. 22 and data not shown). The drastic reduction of β-catenin levels in the high plakoglobin-expressing transfectants indicates the presence of a functional APC pathway in SCC9 cells.

The maintenance of cell number is determined by the balance between cell proliferation and cell death. The results of the TUNEL assays in staurosporine-stimulated low and high plakoglobin cultures suggested that the growth-regulating function of the overexpressed plakoglobin was mediated, at least in part, via its inhibitory effect on programmed cell death. The inhibition of apoptosis was concurrent with the induction of the pro-survival BCL-2 gene expression. There was no detectable level of BCL-2 in the low plakoglobin-expressing transfectants or plakoglobin-deficient SCC9 cells that underwent staurosporine-induced apoptosis.

One mechanism of the anti-apoptotic function of BCL-2 has been shown to operate via its inhibitory effect on activation of the caspase cascade. Caspases are activated by adaptor proteins such as the mammalian Apaf-1 (21, 30). It is suggested that BCL-2 function may be directly binding the adaptor proteins thus inhibiting the activation of the caspase cascade and subsequent execution of the death program. In agreement with this, we only observed a significant decrease in the proteolytic processing of caspase-3 in high plakoglobin-expressing cells which also expressed BCL-2. Interestingly, both plakoglobin and β-catenin can be proteolytically cleaved by caspase-3 during apoptosis, although β-catenin appears to be the in vivo substrate of choice in this process (39, 40).

Taken together, these results demonstrate a role of plakoglobin in regulation of cell growth. The growth regulatory signal induced by plakoglobin, at least in SCC9 cells, appears to function in conjunction with if not via the up-regulation of the anti-apoptotic protein BCL-2 and inhibition of apoptosis. This is in contrast to β-catenin that up-regulates the expression of genes involved in proliferation. Therefore, although both plakoglobin and β-catenin can affect cell number, they may do so by regulating different pathways. Whether plakoglobin directly up-regulates BCL-2 gene expression or indirectly, via modulating the levels of the pro-apoptotic proteins (41), remains to be determined. It is also possible that plakoglobin may exert its growth-regulating effects via down-regulating β-catenin and thus its proposed pro-apoptotic activity. We are currently investigating these alternatives. In either case, plakoglobin appears to be an important component of the molecular mechanisms of adhesion-mediated signaling that control the expression of genes involved in regulating cell growth and malignant transformation.

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REFERENCES

1. Gelderloos, J. A., Witcher, L., Cowin, P., and Klymkowsky, M. W. (1997) in Cytoskeletal-Membrane Interactions and Signal Transduction (Cowin, P., and Klymkowsky, M. W., eds) pp. 13–29, Landes, Austin, TX.

2. Peifer, M., McCrea, P. D., Green, K. J., Wieschaus, E., and Gumbiner, B. M. (1992) J. Cell Biol. 118, 681–691

3. Huber, A., Nelson, W. J., and Weiss, W. (1997) Cell 90, 871–882

4. Chao, T., and Nusse, R. (1998) Curr. Opin. Genet. & Dev. 8, 95–102

5. Rubenstein, A., Merriam, J., and Klymkowsky, M. W. (1997) Dev. Genet. 20, 91–102

6. Bullions, L. C., and Levine, A. J. (1998) Curr. Opin. Oncol. 10, 81–87

7. Simcha, I., Shitutman, M., Salomon, D., Zhurinsky, J., Sadot, E., Geiger, B., and Ben-Ze'ev, A. (1998) J. Cell Biol. 141, 1433–1448

8. Aberle, H., Schwartz, H., and Kemler, R. (1996) J. Cell. Biochem. 61, 514–523

9. Hinck, L., Nathke, I. S., Papoff, H., and Nelson, W. J. (1994) J. Cell Biol. 123, 1327–1340

10. Lewis, J., Wahl, J. K., Ii, S., Jensen, P. J., Johnson, K. R., and Wheelock, M. J. (1997) J. Cell Biol. 136, 919–934

11. Peterkin, E., Verrity, C., and Ben-Ze’ev, A. (1999) J. Cell Biol. 20, 207–229

12. Morin, P. J., Sparks, A. B., Korinek, V., Barker, N., Clevers, H., Vogelstein, B., and Kinzler, K. W. (1997) Science 275, 1778–1780

13. Huber, O., Korn, R., McLaughlin, J., Ohnati, M., Herrmann, B. G., and Kemler, R. (1999) Mech. Dev. 89, 13–18

14. Behrens, J., von Kries, J. P., Kuhl, M., Brief, K., Grosschedl, R., and Birchmeier, W. (1996) Nature 382, 638–642

15. He, T.-C., Sparks, A. B., Rago, C., Hemekeing, H., Zawel, L., da Costa, L. T., Morin, P. J., Vogelstein, B., and Kinzler, K. W. (1998) Science 281, 1509–1512

16. Tetts, O., and McCormick, F. (1999) Nature 396, 429–462

17. Shitutman, M., Zhurinsky, J., Simcha, I., Alband, C., D’Amico, M., Pestell, R., and Ben-Ze’ev, A. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 5522–5527

18. Evan, G., and Littlewood, T. (1998) Science 281, 1317–1322

19. Adams, J. M., and Cory, S. (1998) Science 281, 1322–1326

20. Thornberry, N. A., and Lazebnik, Y. (1998) Science 281, 1312–1316

21. Parker, H. R., Li, Z., Zininov, H., Lauzon, G. J., and Pasdar, J. (1998) Cell Motil. Cytoskeleton 40, 87–100

22. Li, Z., Gallin, W. L., Lauzon, G. J., and Pasdar, J. (1996) J. Cell. Sci. 111, 1005–1019

23. Pasdar, M., Li, Z., and Chumney, V. (1995) Cell Motil. Cytoskeleton 32, 258–272

24. Lu, Q., Abel, P., Foster, C. S., and Lulani, E. N. (1996) Hum. Pathol. 27, 102–110

25. Hermiston, M. L., and Gordon, J. I. (1995) J. Cell Biol. 129, 489–506

26. Alshiba, K., Bernard, S. L., and Sparsym, J. R. (1997) Am. J. Physiol. 272, L28–L37

27. Merio, G. R., and Hynes, N. E. (1997) J. Cell Biol. 138, 251–260

28. Cleary, M. S., Smith, D. D., and Sklar, J. (1986) Cell 47, 19–28

29. Zou, H., Henzel, W. J., Liu, X., Lutschg, A., and Wang, X. (1997) Cell 90, 405–413

30. Behrens, J. (1995) Invasion Metastasis 14, 61–70

31. Kodama, S., Ikeda, S., Asahara, T., Kishida, M., and Kikuchi, A. (1999) J. Biol. Chem. 274, 27682–27688

32. Hart, M. J., de los Santos, R., Albert, L. N., Rubinfeld, B., and Polakos, P. (1998) Curr. Biol. 8, 573–581

33. Sakurak, C., Weiss, J. B., and Williams, L. T. (1998) Proc. Natl. Acad. Sci.
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Aberle, H., Bauer, A. Stappert, J., Kispert, A., and Kemler, R. (1997) EMBO J. 16, 3797–3804
Salomon, D., Sacco, P. A., Roy, S. G., Simcha, I., Johnson, K. R., Wheelock, M. J., and Ben-Ze’ev, A. (1997) J. Cell Biol. 139, 1325–1336
Ahmad, Y., Hayashi, S., Levine, A., and Wieschaus, E. (1998) Cell 93, 1171–1182
Strater, J., Koretz, K., Gunthert, A. R., and Möller, P. (1995) Gut 37, 819–825
Herren, B., Levkau, B., Raines, E. W., and Ross, R. (1998) Mol. Biol. Cell 9, 1589–1601
Brancolini, C., Sgorbissa, A., and Schneider, C. (1998) Cell Death Differ. 5, 1042–1050
Oltvai, Z. N., Milliman, C. L., and Korsmeyer, S. J. (1993) Cell 74, 609–619
Domen, J., Gandy, K. L., and Weissman, I. L. (1998) Blood 91, 2272–2282
Plakoglobin Regulates the Expression of the Anti-apoptotic Protein BCL-2
Shahram Hakimelahi, Henry R. Parker, Anita J. Gilchrist, Michele Barry, Zhi Li, R. Chris Bleackley and Manijeh Pasdar

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