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Relationship of the Feline Oncornavirus Associated Cell Membrane Antigen to a Feline Sarcoma Virus Encoded Polyprotein

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A. Introduction

The feline oncornavirus associated cell membrane antigen (FOCMA) is expressed on the membranes of cells transformed by infection with the feline leukemia virus (FOCMA-L) or feline sarcoma virus (FOCMA-S) (Hardy et al. 1977; Essex et al. 1977). Expression of FOCMA is transformation-specific and not dependent upon concomitant expression of antigens associated with feline leukemia virus (FeLV) or the endogenous virus RD114 (reviewed in Snyder et al., to be published a). In the natural environment FOCMA is the target for an effective immunosurveillance response: complement-dependent lytic antibodies directed to FOCMA reverse or prevent tumor development (Essex et al. 1975; Grant et al. 1977). FOCMA-L has been isolated from feline lymphosarcoma (LSA) cell membranes and has been shown to reside on a 70,000 dalton protein which is neither glycosylated nor phosphorylated (Snyder et al. 1978, to be published a). The nature of FOCMA-S is a subject of intense investigation at the present time.

The observation that FOCMA is induced on FeSV-transformed nonproducer fibroblasts of nonfeline as well as feline origin has been interpreted as evidence that FOCMA-S may actually be encoded by FeSV (Essex et al. 1979). Present evidence suggests that in the derivation of the defective FeSV genome sequences from the *pol* and the 3′ end of the *gag* and *env* genes were deleted and the *gag-pol* deletion was substituted with cat cellular DNA sequences which contain information for transformation (Frankel et al. 1979; Sherr et al. 1980). The translation products of FeSV genomes in nonproducer fibroblasts are "fusion" proteins comprised of covalently linked FeLV p15, p12, a portion of p30, and nonstructural ("x") components of differing sizes depending on the particular strain of FeSV (Khan and Stephenson 1977; Sherr et al. 1978a; Barbacid et al. 1980; Van de Ven et al. 1980; Ruscetti et al., to be published). These proteins have generally been referred to as "gag-x" polyproteins. The question of FOCMA association with the "x" portion of these fusion proteins is addressed below.

B. Results

We have previously reported the isolation of 70,000 dalton proteins from lysates of feline LSA cells by immunoaffinity chromatography on a column of Sepharose containing bound IgG from cat anti-FOCMA serum (Snyder et al., to be published a; Fig. 1A). Using the same affinity column we isolated a similarly sized protein from lysates of mink cells transformed by the Gardner-Arnstein strain of FeSV (GA-FeSV) (Fig. 1, B). This result was unanticipated since published evidence suggested an association between FOCMA and the much larger (95,000 daltons) gag-x fusion protein in these cells (Stephenson et al. 1977; Sherr et al. 1978a,b; Snyder et al. 1978). In addition to the p70 it was possible to isolate the Ga-FeSV fusion protein (termed $P95^{gag\text{-}x}$) from the transformed mink cells by virtue of its binding to anti-FeLV antibodies on a different affinity column (Fig. 1, C).

These purified proteins as well as those directly immunoprecipitated from detergent-lysed cultured cells were analyzed for (1) associated FeLV antigens by radioimmunopre-

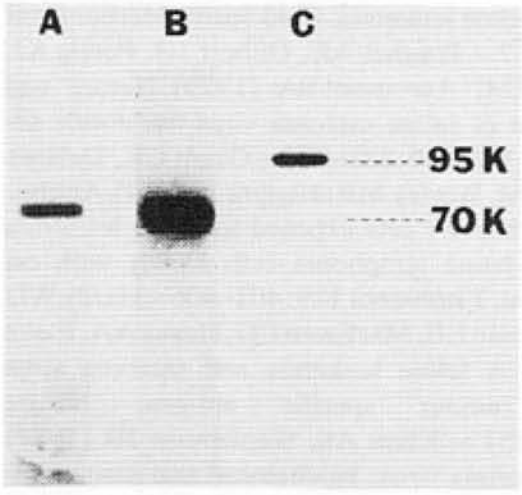

Fig. 1. SDS polyacrylamide gel electrophoresis of *(A)* FOCMA-L molecules purified by immunoaffinity chromatography of a feline LSA (FeLV nonproducer) over a column of Sepharose containing bound IgG from cat FOCMA serum, *(B)* FOCMA-S molecules purified over the same column from a lysate of GA-FeSV mink cells (64F3C17 cells), and *(C)* $P95^{gag\text{-}x}$ molecules purified on an anti-FeLV IgG immunoaffinity column from a lysate of 64F3C17 cells. Aliquots of material binding to the columns and eluted with 4 *M* $MgCl_2$ were radioiodinated and subjected to electrophoresis in a 5%–20% acrylamide slab gel and visualized by autoradiography.

cipitation with specific antisera to individual proteins, (2) glycosylation and phosphorylation (Snyder et al., to be published a), (3) similarities in peptides generated by partial digestion with *S. aureus* V8 protease or chymotrypsin (Snyder et al., to be published b), (4) associated protein kinase activity (Collett and Erikson 1978; Sen and Todaro 1979; Snyder et al., to be published b), and (5) associated glucose binding and/or transport-stimulating activity (Lee and Lipmann 1977). In terms of this preliminary characterization (Table 1) the purified p70 molecules from LSA cells and GA-FeSV-transformed mink fibroblasts were indistinguishable. However, the gag-x polyprotein was distinguishable by its labeling with ^{32}P-orthophosphate, its association with a protein kinase activity, and its precipitability with antibodies in a hyperimmune antiserum to FeLV structural proteins but not with antibodies in a well-characterized cat-derived natural FOCMA serum.

C. Discussion

Previously, two independent investigators showed that absorption of certain cat FOCMA sera with FeLV structural proteins did not diminish immunoflourescent antibody reactivity for FOCMA-S on FeSV-transformed mink cells, while absorption with partially-purified gag-x polyproteins was effective (Stephenson et al. 1977; Sherr et al. 1978a). This was the most direct evidence for a link between FOCMA and the "x" portion of the FeSV polyprotein. Indirect supportive evidence came from an experiment wherein hyperimmune rabbit antibody to FeSV gag-x-containing pseudotype virions, made specific for "x" by absorption with helper virus proteins, stained the surface of FL 74 LSA cells (Sherr et al. 1978b). Khan et al. (1978) showed that many FeLV-absorbed cat FOCMA sera were capa-

Table 1. Comparison of proteins induced in cells transformed by FeLV and FeSV

	FOCMA-L p70	GA-FeSV	
		gag-x	p70
Cell localization	Plasma membrane	Plasma membrane	Plasma membrane
Molecular weight	65–70K	95K	65–70K
Associated FeLV Ag	None	p15p12(p30)	None
Protease digest maps	Similar	?	Similar
Phosphorylation	−	+	−
Glycosylation	−	−	−
Associated protein Kinase activity	No	Yes	No
Associated glucose Binding activity	No	?	No
Associated glucose transport stimulating activity	No	?	No

ble of reacting in sensitive radioimmunoprecipitation assays with a highly purified gag-x protein probe. However, the fact that not all such sera displayed this reactivity was the first suggestion that there may not be complete concordance between recognition of FOCMA and recognition of "x". Recent experiments by Barbacid et al. (1980) and Ruscetti et al. (to be published) as well as those described in the present report are consistent with this lack of complete concordance. While we have not ruled out the possibility that the p70 molecule we have isolated from GA-FeSV-transformed mink cells is a breakdown product of unstable $P95^{gag\text{-}x}$, for the present p70 appears to be a good alternate candidate for a FOCMA-bearing protein in these cells. By extrapolation from all of the available evidence one might speculate that FOCMA, as defined in terms of a tumor regression-correlated cat antibody reaction with the surface of FL74 LSA cells, may actually be a family of antigens, not all of which cross react with FeSV polyprotein antigens.

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References

Barbacid M, Lauver AV, Devare SG (1980) Biochemical and immunological characterization of polyproteins coded for by the McDonough, Gardner-Arnstein, and Snyder-Theilen strains of feline sarcoma virus. J Virol 33:196–207 – Collett MS, Erikson RL (1978) Protein kinase activity associated with the avian sarcoma virus src gene product. Proc Natl Acad Sci USA 75:2021–2024 – Essex M, Sliski A, Cotter SM, Jakowski RM, Hardy WD Jr (1975) Immunosurveillance of naturally occurring feline leukemia. Science 190:790–792 – Essex M, Cotter SM, Stephenson JR, Aaronson SA, Hardy WD Jr (1977) Leukemia, lymphoma, and fibrosarcoma of cats as models for similar diseases of man. Cold Spring Harbor Conf Cell Prolif 4:1197–1214 – Essex M, Sliski AH, Worley M, Grant CK, Snyder HW Jr, Hardy WD Jr, Chen LB (to be published) Significance of the feline oncornavirus-associated cell membrane antigen (FOCMA) in the natural history of feline leukemia. Cold Spring Harbor Conf Cell Prolif 7 – Frankel AE, Gilbert JH, Porzig KJ, Scolnick EM, Aaronson SA (1979) Nature and distribution of feline sarcoma virus nucleotide sequences. J Virol 30:821–827 – Grant CK, DeBoer DJ, Essex M, Worley MB, Higgins J (1977) Antibodies from healthy cats exposed to feline leukemia virus lyse feline lymphoma cells slowly with cat complement. J Immunol 119:401–406 – Hardy WD Jr, Zuckerman EE, MacEwen EG, Hayes AA, Essex M (1977) A feline leukemia and sarcoma virus induced tumour specific antigen. Nature 270:249–251 – Khan AS, Stephenson JR (1977) Feline leukemia virus: biochemical and immunological characterization of gag gene-coded structural proteins. J Virol 23:599–607 – Khan AS, Deobagkar DN, Stephenson JR (1978) Isolation and characterization of a feline sarcoma virus-coded precursor polyprotein: competition immunoassay of nonstructural components. J Biol Chem 253:8894–8901 – Lee SG, Lipmann F (1977) Isolation from normal and Rous sarcoma virus-transformed chicken fibroblasts of a factor that binds glucose and stimulates its transport. Proc Natl Acad Sci USA 74:163–166 – Ruscetti SK, Donner L, Sherr CJ (to be published) A model for FOCMA expression in cells transformed by feline leukemia and sarcoma viruses. In: Fields B, Jaenisch R, Fox CF (eds) ICN-UCLA symposia on molecular and cellular biology, Vol 18. Academic Press, New York – Sen A, Todaro GJ (1979) A murine sarcoma virus-associated protein kinase: interaction with actin and microtubular protein. Cell 17:347–356 – Sherr CJ, Sen A, Todaro GJ, Sliski A, Essex M (1978a) Feline sarcoma virus particles contain a phosphorylated polyprotein (pp85) with p15, p12 and FOCMA antigens. Proc Natl Acad Sci USA 75:1505–1509 – Sherr CJ, Todaro GJ, Sliski A, Essex M (1978b) Characterization of a feline sarcoma virus-coded antigen (FOCMA-S) by radioimmunoassay. Proc Natl Acad Sci USA 75:4489–4493 – Sherr CJ, Fedele LA, Oskarsson M, Maizel J, Vande Woude G (1980) Molecular cloning of Snyder-Theilen feline leukemia and sarcoma viruses: comparative studies of feline sarcoma virus with its natural helper virus and with Moloney sarcoma virus. J Virol 34:200–212 – Snyder HW Jr, Hardy WD Jr, Zuckerman EE, Fleissner E (1978) Characterization of a tumour-specific antigen on the surface of feline lymphosarcoma cells. Nature 275:656–658 – Snyder HW Jr, Phillips KJ, Hardy WD Jr, Zuckerman EE, Essex M, Sliski AH, Rhim J (to be published a) Isolation and characterization of proteins carrying the feline oncornavirus-associated cell membrane antigen (FOCMA). Cold Spring Harbor Symp Quant Biol 44 – Snyder HW Jr, Phillips KJ, Dutta-Choudhury M, Hardy WD Jr, Zuckerman EE (to be published b) Biochemical and serological aspects of proteins induced in cells transformed by FeLV and FeSV. In: Hardy WD Jr, Essex M, McClelland AJ (eds) Feline leukemia virus. Elsevier, New York – Stephenson JR, Khan

AS, Sliski AH, Essex M (1977) Feline oncornavirus-associated cell membrane antigen (FOCMA): identification of an immunologically cross-reactive feline sarcoma virus-coded protein. Proc Natl Acad Sci USA 74:5608–5612 – Van de Ven WJM, Khan AS, Reynolds FH Jr, Mason KT, Stephenson JR (1980) Translational products encoded by newly acquired sequences of independently derived feline sarcoma virus isolates are structurally related. J Virol 33:1034–1045