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Original Research Article

Comparative Expression of NFκB Proteins in Melanocytes of Normal Skin vs. Benign Intradermal Naevus and Human Metastatic Melanoma Biopsies

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Nuclear factor kappa B (NFκB) is an essential regulator of gene transcription for hundreds of genes, including many critically involved in apoptosis. NFκB complexes containing cRel generally activate pro-apoptotic genes, while those with RelA activate anti-apoptotic genes. We have previously shown that NFκB binding by RelA is constitutively elevated in human metastatic melanoma cultures relative to normal melanocytes. Here we extended our investigation to immunohistochemical analysis of human tissue biopsies. We found that RelA expression is significantly elevated in melanocytes of human naevi and melanomas relative to normal skin, but expression of its inhibitor IκB-α is significantly lower in metastatic melanomas than in intradermal naevi. Antibodies specific for the nuclear localization signal of RelA also showed significantly increased staining in metastatic melanoma biopsies. Notably, in melanomas and in naevi, we also found that RelA is phosphorylated at serine 529, and this activated form accumulates in the nuclei of melanomas. This suggests that increased expression and phosphorylation of RelA occurs at the stage of the benign naevus, but IκB-α is able to sequester RelA in the cytoplasm and regulate RelA transcriptional transactivation. We also found that antibodies against cRel show a progressive increase in staining from naevi to melanoma. However, staining for IκB-ε, which primarily inhibits the nuclear localization of cRel was also progressively increased, and cRel expression was predominantly cytoplasmic in melanomas. These results confirm that the altered expression of RelA found in metastatic melanoma cells in tissue culture is relevant to human tumors and offer new insights into the deregulation of NFκB signaling.

Key words: nuclear factor kappa B, RelA, cRel, inhibitor of kappa B-α, inhibitor of kappa B-β, inhibitor of kappa B-ε

INTRODUCTION

The incidence of melanoma is increasing at the second highest rate of all human cancers, and metastatic disease is generally fatal (1, 2). Metastatic melanomas are typically resistant to radiation and chemotherapy (1, 3), suggesting that some melanomas maintain an anti-apoptotic phenotype. Currently, there is a critical need for the development of therapeutic agents that could target aberrant survival pathways in metastatic melanomas. Recently, the role of the transcription factor nuclear factor kappa B (NFκB) in protecting melanoma cells from apoptosis has received considerable interest. We (4, 5) and others (6, 7) have shown that NFκB is constitutively activated in metastatic melanoma cells in culture.

The mammalian NFκB family contains five members; p105/p50, p100/p52, RelA, RelB and cRel that share a highly conserved 300 amino acid Rel homology domain with dimerization, nuclear localization and DNA binding regions (8). RelA, RelB and cRel also contain transcriptional transactivation domains that directly activate expression of over 100 genes, including many critical in apoptosis. RelA, cRel, p105/p50 and p100/p52 can form multiple homo- and hetero-dimers, each of which has distinct DNA binding site specificities and affinities (9–13). Upon activation, these dimers translocate to the nucleus where they bind DNA at NFκB sequences found in the promoters of target genes (8, 13–15).

Abbreviations – cIAP, cellular inhibitor of apoptosis; CK2, formerly known as casein kinase II; DR, death receptor; HDAC-1, histone deacetylase-1; IκB, inhibitor of kappa B; IKK, inhibitor of kappa kinase; NFκB, nuclear factor kappa B; NLS, nuclear localization signal; TRAIL, TNF-related apoptosis-inducing ligand

The dimeric composition of NFκB is critical to the specificity of gene transactivation. cRel containing NFκB dimers can transactivate different genes than those dimers containing RelA (15, 16). For example, RelA inhibits expression of the TNF-related apoptosis-inducing ligand (TRAIL) receptors DR4/DR5 and up-regulates cIAP1, cIAP2 and caspase-8. Conversely, c-Rel enhances expression of DR4/DR5, Bcl-X and inhibits cIAP1, cIAP2, and survivin after TRAIL treatment. Whether NFκB functions as an inhibitor or activator of apoptosis depends on the relative levels of RelA and c-Rel subunits (15). Transcriptionally inactive nuclear NFκB in resting cells consists of homodimers of either RelA or p50 complexed with the histone deacetylase-1 (HDAC-1). RelA homodimers complexed with HDAC-1 have low DNA binding affinity, but p50 homodimers lacking transactivation domains bind DNA with high affinity and are transcriptionally repressive (17).

NFκB regulation is mediated through multiple kinase pathways that phosphorylate precursors (p105, p100), inhibitory proteins (IκB) and RelA (17–19). The three major IκB proteins have distinct binding affinities for the various homo- and hetero-dimeric NFκB complexes (8). IκB-α preferentially inhibits p50 heterodimers containing RelA and to a lesser extent those with cRel. Unphosphorylated IκB-β preferentially inhibits RelA homo- and hetero-dimers but phosphorylated IκB-β preferentially binds cRel dimers. IκB-ε is associated with cRel and RelA, but not with p50 (8, 14, 16, 20, 21).

In general, IκB-α is more effective in inhibition of nuclear NFκB complexes than IκB-β, but is rapidly degraded by the proteasome in response to stimuli (22). IκB-β is less sensitive to stimulus dependent degradation (13). Upon activation, IκB-ε protein is also degraded with slow kinetics by a proteasome-dependent mechanism that appears to regulate a late, transient activation of a subset of genes, regulated by RelA/cRel complexes (16).

Activation of NFκB leads to the immediate transcriptional transactivation of its inhibitors p100, p105 and IκB-α. Induction of newly synthesized inhibitors by NFκB functions as an immediate negative feedback mechanism that leads to sequestration of the transactivating NFκB as inactive cytoplasmic complexes. Induction of IκB-α in particular facilitates export of activated RelA-p50 heterodimeric complexes from the nucleus thereby terminating NFκB function (8, 16, 20, 23–26).

The specificity of NFκB signaling is not only fine tuned by its homo- and hetero-dimeric composition. There are multiple levels of regulation of NFκB activation, including phosphorylation and acetylation of NFκB proteins, phosphorylation and acetylation dependent recruitment of transcriptional coactivators and subsequent modification of nucleosomal histones (13).

The expression, localization and signaling of specific NFκB subunits and inhibitors has been studied in detail in immune cells and in malignancies (13). NFκB expression and signaling in normal melanocytes, benign naevi and malignant melanoma however is not well detailed. Elevated inhibitor of kappa kinase (IKK) activity, enhanced degradation of IκB-α and constitutive activation of NFκB have been shown in Hs294T melanoma cells (6, 7, 27) and NFκB activity was shown to correlate with angiogenesis and metastasis of human melanoma in a nude mouse model (28). We have shown that NFκB DNA binding mediated by RelA-p50 dimers is constitutively elevated in human metastatic melanoma cell cultures and that nuclear and cytoplasmic levels of RelA protein are elevated in melanoma cells (4). RelA RNA expression is also elevated in metastatic melanoma relative to normal human melanocyte cultures (4, 5).

To investigate the expression pattern of NFκB family members in human melanocytic cells of normal skin, benign intradermal naevus and metastatic melanoma tissues, we analyzed 60 human biopsies (20 normal skin, 20 benign naevus and 20 metastatic melanoma) for expression of RelA, cRel, p105/p50, and the inhibitory proteins IκB-α, IκB-β and IκB-ε. We also examined the expression of an activated form of RelA, serine 529-phosphorylated RelA.

MATERIALS AND METHODS

Immunohistochemistry

All metastatic melanoma and benign intradermal naevus diagnoses on tissues used in these studies were made independently by pathologists not involved in this study. Normal skin biopsies were obtained from amputations. All of the naevi were classified as benign, no junctional or dysplastic naevi were included in this analysis. Formalin-fixed, paraffin-embedded sections from normal skin, benign naevus and metastatic melanoma biopsies were placed on capillary gap slides, deparaffinized with Histoclear and rehydrated through decreasing concentrations of isopropyl alcohol. Mounted tissues were immersed in 0.25% $KMnO_4$ for 10 min, rinsed in water, immersed in 5% oxalic acid until cleared (< 1 min) and rinsed in water prior to staining to bleach melanin (29). All antibodies used were determined to be compatible with the bleaching process. Avidin-biotin complex (ABC) immunoperoxidase reactions were performed using an Immunotech 500 automated immunostainer (Ventana Systems Inc., Tucson, AZ, USA) according to the manufacturer's instructions. Briefly, the automated steps included antigen retrieval by steam, blockage of endogenous peroxidase with 3% hydrogen peroxide and incubation with the primary antibody. The reaction was followed by a biotinylated goat-antimouse IgG secondary antibody and then an avidin–biotin peroxidase complex. The chromogen was diaminobenzidine for all reactions. Negative controls, in which slides were treated as above, with the exception of the addition of the primary antibody, were run simultaneously. Identification of melanocytic cells in human skin, naevus and melanoma sections was done by use of initial hematoxylin–eosin stain (30). Melanocytes are recognizable as randomly dispersed cells, wedged between the basal cells within the epidermis. Melanocytes have dark-staining nuclei and largely as a result of retraction or shrinkage, clear cytoplasm. Not all clear cells in the sections examined were melanocytes. Occasionally, basal keratinocytes show some shrinkage artifact, in these cases intercellular bridges were used to differentiate basal keratinocytes with cytoplasmic clearing from melanocytes. In instances, where it was not possible to

differentiate between keratinocytes and melanocytes, the cells in question were not scored. Only melanocytic cells, as determined by the dermatopathologist involved in this study, were included in the analysis.

Statistical Analysis

Cells were scored by intensity of immunoperoxidase staining as negative (0), or positive with a grade of 1+, 2+ or 3+. Cells of melanocytic origin scored as 2+ or 3+ were rated with high positivity and the mean ± SD was calculated using Prism GraphPad for each group: skin, naevus or metastatic melanoma. Student's *t*-test and one-way analysis of variance with the Bonferroni Multiple Comparison Test and were performed to determine significant differences (defined as $P < 0.05$).

Antibodies

Antibodies directed against specific NFκB proteins were obtained from Nancy Rice (NIH Bethesda, MD, USA), p105/p50 (PAb-1157, 1:5000); Rockland Immunochemicals (Gibertsville, PA, USA), serine 529-phosphorylated RelA (PAb-200-3165, 1:500); Chemicon International (Temecula, CA, USA), nuclear localization specific RelA (Mab-3026, 1:2000); Santa Cruz Biotech (Santa Cruz, CA, USA), RelA (MAb-sc-8008, 1:10 000), cRel (MAb-sc-6955, 1:250) IκB-α (PAb-sc-371, 1:2000) Iκ-β (PAb-sc-945, 1:500), Iκ-ε (PAb-sc 7155, 1:500).

RESULTS

RelA Expression is Elevated in Naevus and Melanoma Biopsies

The percentages of melanocytic cells with high positive staining for each group; normal skin melanocytes, benign intradermal naevi and metastatic melanoma are shown in Fig. 1. Representative sections of each group are shown in Figs 2 and 3. Using primary antibodies specific for RelA, we found that melanocytes in normal skin had low expression of RelA [Figs 1A and 2 (1A)]. Although the melanomas showed

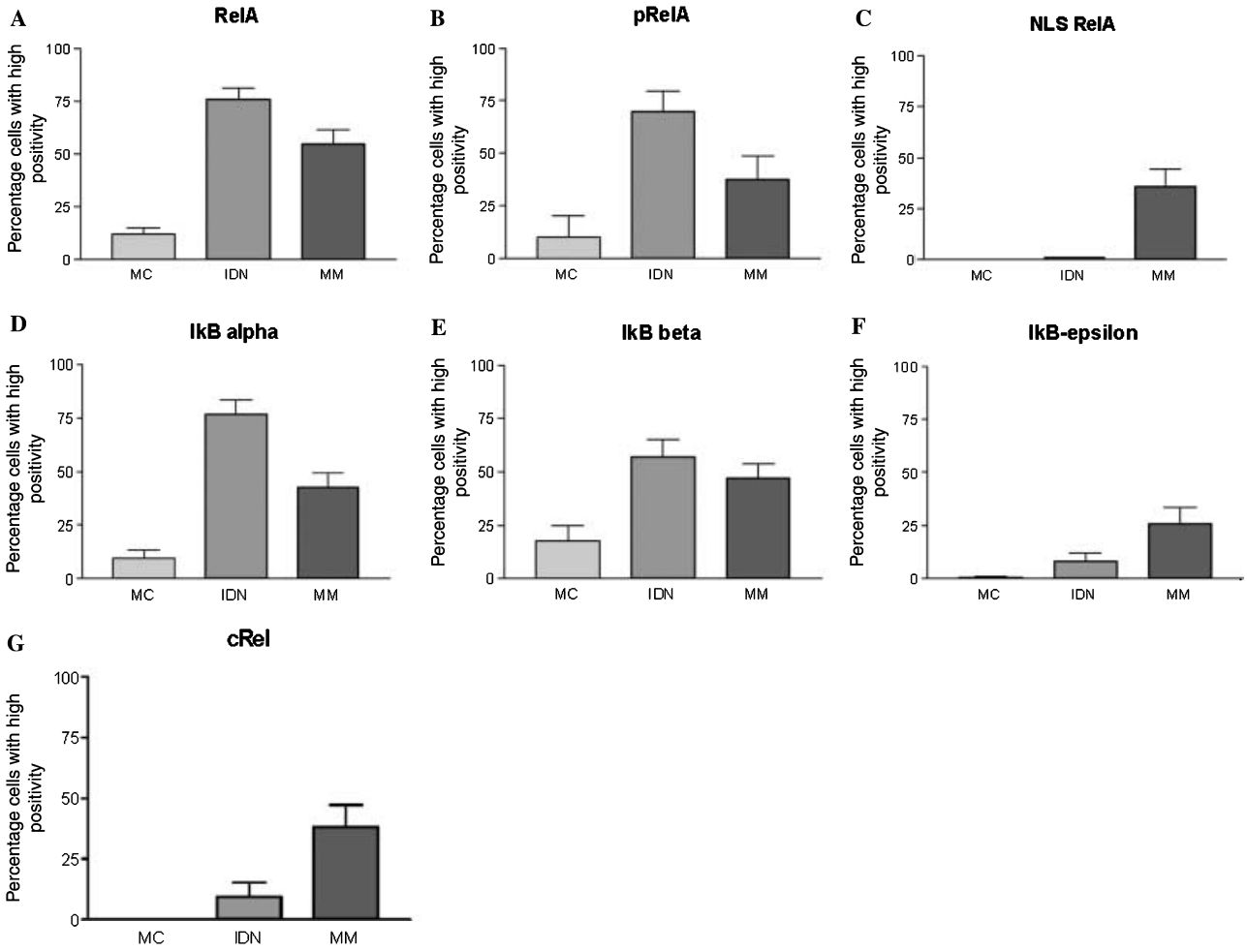

Fig. 1. Relative expression of NFκB proteins in melanocytes of normal skin, benign naevus and metastatic melanoma biopsies. The percentage (±SD) of melanocytic cells in normal skin (MC), benign intradermal naevus (IDN) and metastatic melanoma (MM) biopsies staining with high positivity for each antibody directed against: (**A**) RelA, (**B**) phosphoserine-529 RelA (pRelA), (**C**) nuclear localization signal specific RelA (NLS RelA), (**D**) IκB-α, (**E**) IκB-β (**F**) IκB-ε, (**G**) cRel.

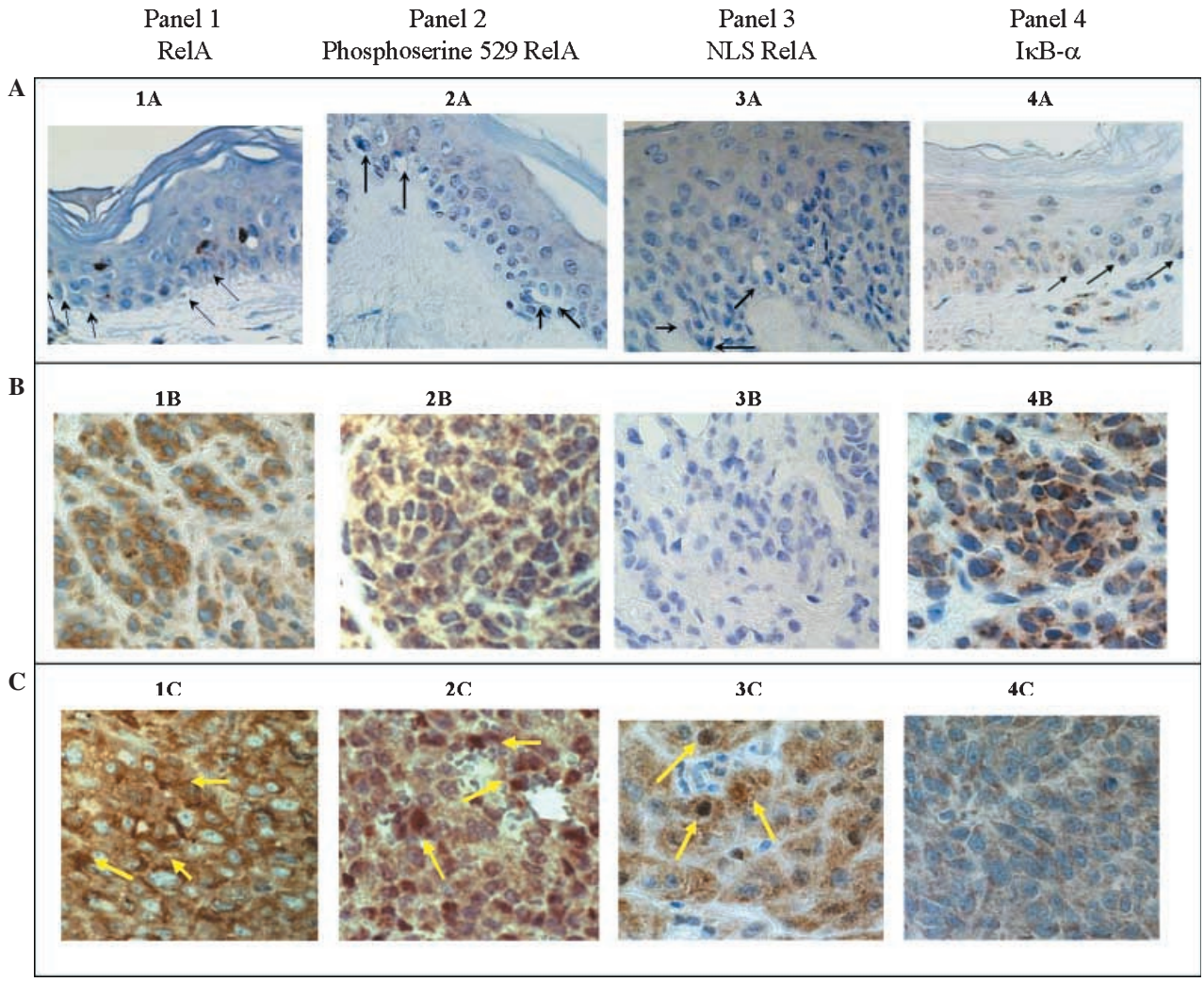

Fig. 2. Expression of RelA and IκB-α. Paraffin embedded sections from (**A**) normal skin, (**B**) benign naevus and (**C**) metastatic melanoma biopsies were stained as described in Materials and methods. Positive immunoperoxidase staining appears as brown regions of the cells. Unstained nuclei appear as central blue regions, positive nuclei are seen as central brown regions and are indicated by yellow arrows. In the normal skin biopsies, melanocytes at the dermal border are indicated by black arrows. In the skin biopsies, keratinocytes and dendritic cells in the upper epidermal layer are also visible. In the melanoma biopsies, infiltrating lymphocytes are visible. However, only cells of melanocytic origin were included in the analysis. The primary antibodies used were panels (1) Anti-RelA antibody, (2) Anti-phosphoserine-529 specific RelA antibody, (3) Anti-nuclear localization signal specific RelA antibody (NLS RelA), (4) Anti-IκB-alpha antibody, 40× magnification.

greater heterogeneity both within and among biopsies, significantly higher percentages of melanocytic cells from naevus and metastatic melanoma biopsies had high positivity for RelA compared with melanocytes in normal skin biopsies ($P < 0.05$), as shown in Figs 1A and 2 (1A, 1B, 1C).

Phosphoserine-529 RelA is Elevated in Naevus and Melanoma Biopsies and is Both Cytoplasmic and Nuclear in Metastatic Melanomas

Using primary antibodies specific for phosphoserine-529 RelA (pRelA), we found that significantly higher percentages of melanocytic cells from naevus and metastatic melanoma biopsies had high positivity for pRelA compared with melanocytes in normal skin biopsies ($P < 0.05$), as shown in Fig. 1B. One hundred percent of the naevus and 80% of the melanoma biopsies expressed high levels of phosphoserine-529 RelA, as shown in Figs 1B and 2 (2B, 2C). In contrast, only 10% of the normal skin biopsies had melanocytes that expressed high (2+) levels of this phosphorylated form of RelA [Figs 1B and 2 (2A)].

Nuclear expression of phosphoserine-529 RelA was significantly higher in melanoma than in naevi ($P < 0.001$). As shown in Fig. 2 (2B), expression of phosphoserine-529 RelA was almost exclusively cytoplasmic in naevi. However, 40% of the melanoma biopsies also expressed high nuclear levels of phosphoserine-529 RelA [Fig. 2 (2C)].

The Nuclear Localization Signal of RelA is Unmasked in Melanomas

As shown in Figs 1C and 2 (3A, 3B, 3C), the percentage of cells with high positivity for the unmasked nuclear localization

signal (NLS) domain of RelA was significantly higher in melanoma cells than in melanocytes from either skin or naevus biopsies ($P < 0.001$). Furthermore, 3+ levels of this form were detected exclusively in metastatic melanomas (data not shown).

Expression of IκB Proteins is Increased in Both Naevi and Melanomas; IκB-β Expression is Raised to Equivalent Levels, But IκB-α is Higher While IκB-ε is Lower in Naevi than in Melanomas

As shown in Figs 1D and 2 (4A, 4B, 4C), both naevus and melanoma biopsies had a greater percentage of melanocytic cells that showed high positivity for the inhibitory protein IκB-α than melanocytes in normal skin biopsies ($P < 0.001$ and $P < 0.05$, respectively). However, the percentage of cells with high positive staining with IκB-α antibodies was significantly reduced ($P < 0.05$) in melanomas relative to naevi and there was also a loss in the intensity of immunostaining in melanomas compared with naevi (data not shown). Furthermore, all of the melanoma biopsies that showed high nuclear expression of phosphoserine 529-RelA also expressed low levels of the inhibitor IκB-α. As seen in Fig. 1E, IκB-β was elevated equally in naevi and in melanomas. However, as shown in Fig. 1F, IκB-ε was elevated to a greater extent in melanomas than in naevi.

cRel is Increased in Melanomas and Correlates with Increased IκB-ε Expression

As shown in Fig. 1G, the percentage of melanoma cells showing high positivity for cRel was significantly higher in melanomas than the percentage of melanocytes either from naevus or from normal skin biopsies ($P < 0.001$). About 60% of the melanoma biopsies exhibited high levels of cRel in comparison with 0% of normal skin biopsies and 15% of naevus biopsies [Fig. 3 (1A, 1B, 1C)]. There was also a shift in the percentage of cells with 2+ to those with 3+ in the intensity of cRel immunostaining in melanomas compared with naevi (data not shown). Of the melanoma biopsies with cells that showed high positivity for cRel, 80% also expressed high levels of IκB-β, 50% expressed high levels of Iκ-Bε, 90% expressed high levels of either IκB-β or Iκ-Bε and 50% expressed high levels of both of these inhibitory proteins. While 90% of the melanoma biopsies were negative for nuclear cRel, one had less than 1% of cells with nuclear cRel expression, but one had 30% nuclear cRel staining. In this biopsy, 90% of the cells had low expression of IκB-β and 70% had low IκB-ε.

p50 was highly expressed in all cells in normal skin, benign naevus and metastatic melanoma biopsies. Because intercellular bridges could not be unambiguously determined in the normal skin tissues stained with p50 antibodies, statistical

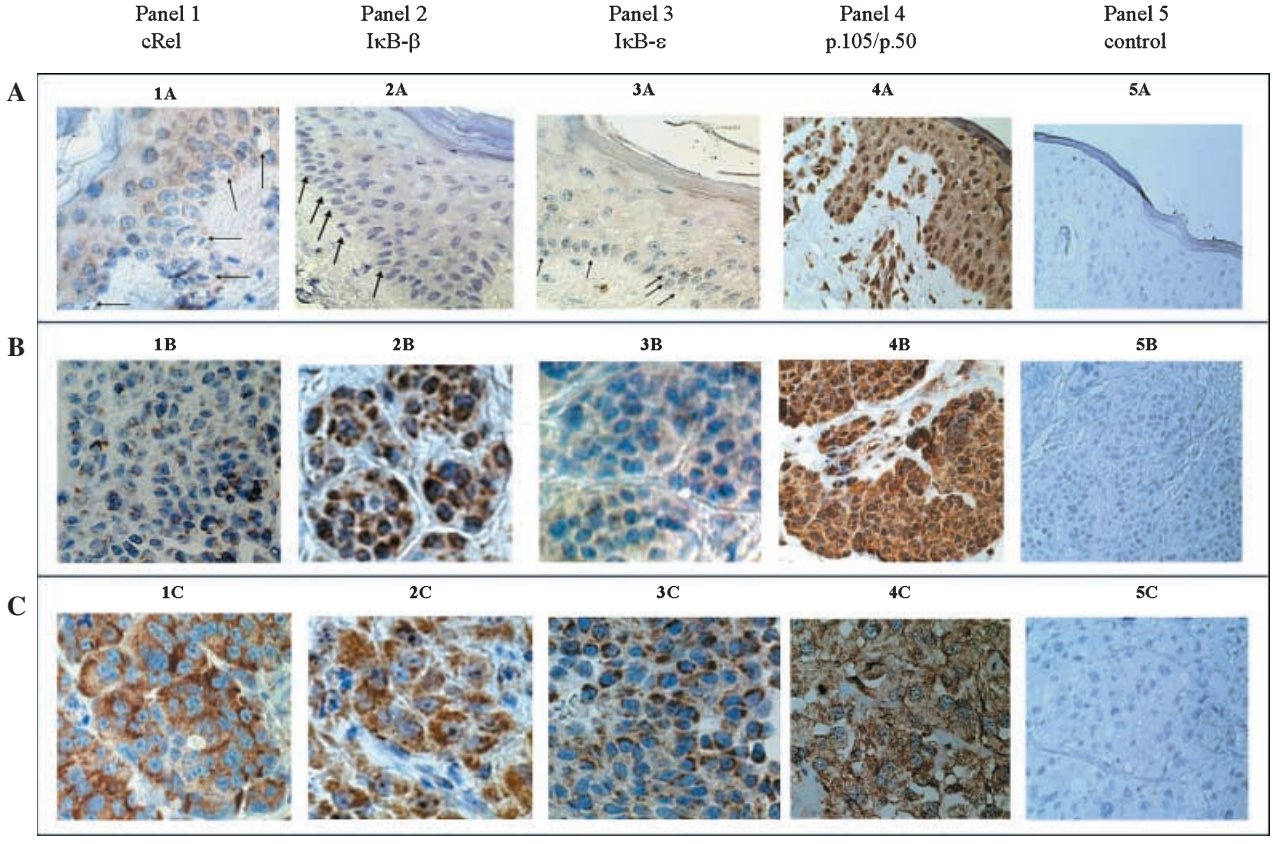

Fig. 3. Expression of cRel, IκB-β, IκB-ε, and p105/p50. Paraffin embedded sections from (**A**) normal skin, (**B**) benign naevus and (**C**) metastatic melanoma biopsies are as described in Fig. 2. Panels (1) anti-cRel antibody, (2) anti-IκB-β antibody, (3) anti-IκB-ε antibody, (4) anti-p105/p50 antibody, (5) negative controls, 40× magnification.

analysis was not performed and p50 data were presented qualitatively. Cytoplasmic p50 was also highly expressed in the metastatic melanoma biopsies, but the nuclear p50 appeared to be less intense, as shown in Fig. 3 (4A, 4B, 4C). Negative controls, which lacked the relevant primary antibody, were run simultaneously, and as shown in Fig. 3 (5A, 5B, 5C), cells were negative for immunoperoxidase staining.

DISCUSSION

Studies previously conducted in tissue culture showed that RelA expression and DNA binding was higher in metastatic melanoma cells than in melanocytes (4). In this study, we found that the melanoma cells in the majority of patient biopsies also had significantly higher expression of RelA than melanocytes found in normal epidermal tissues. Surprisingly, we also found that RelA expression was significantly higher in the melanocytes found in benign intradermal naevus biopsies compared with melanocytes found in normal epidermal skin tissues.

It is interesting to note that in two of the melanoma biopsies, the melanoma cells had a distinctly spindled morphology and expressed low levels of RelA (0/+1) in contrast to the other melanoma biopsies. Desmoplastic malignant melanoma (DMM) is a rare variant of malignant melanoma histologically distinguishable by its spindle cells. Although distant metastases do develop, DMM typically presents with nodal metastasis less frequently than other forms of melanoma, and has a better prognosis. DMM has a tendency for local recurrence after apparently complete surgical excision, but this can be dramatically reduced by adjuvant radiation therapy (31).

We could speculate that alterations in RelA expression may be correlated with melanomas that arise in a naevus, but perhaps not those that arise independently. Future analyses of primary melanomas by depth, association with a naevus and classification (superficial spreading, nodular, desmoplastic, lentiginous, accral) may help to clarify this. Because RelA is known to protect cells against apoptosis from radiation and chemotherapy (32–36), RelA expression levels may help in predicting whether a particular melanoma will respond to radiation therapy. These observations warrant further investigation.

We also found that serine-529 phosphorylated RelA is elevated in melanoma and naevus biopsies relative to melanocytes from normal skin. However, significantly more of this activated form of RelA accumulated in the nuclear compartment of melanomas than naevi. In addition, we found a significant increase in expression of RelA with an unmasked NLS in melanoma biopsies relative to melanocytes in both normal skin and naevus biopsies. Dhawan et al. also reported that NFκB activation, which they measured as expression of the unmasked NLS, was associated with Akt/PKB in melanoma and dysplastic naevus (37). They further reported that inhibition of PI3 kinase and Akt corresponded with a decrease in NFκB-luciferase reporter assays but showed no effect on IKK activity (37). Akt has been shown by others to activate the transactivation potential of RelA without increasing IκB-α degradation or nuclear localization of RelA (38–40). Recent studies have shown that Akt is a downstream target of NFκB and that nuclear translocation of RelA and increased NFκB DNA binding activity precede Akt phosphorylation (38). Furthermore, over-expression of RelA causes an increase in Akt mRNA and protein and stimulates phosphorylation of Akt (38, 41).

Multiple kinase pathways phosphorylate NFκB inhibitory proteins and precursors as well as RelA. The catalytic subunit of protein kinase A phosphorylates RelA at serine-276 (19, 42) and PKCζ phosphorylates RelA at serine 311 (43). These phosphorylation events stimulate the transactivation potential of RelA by facilitating recruitment of the coactivators CBP/p300 (17, 19, 38, 41, 43). It has been proposed that Akt also stimulates the transactivation potential of RelA by CBP/p300 interactions (44, 45). The IKK complex controls stimulus dependant degradation of IκB-α (18, 46–48) and RelA is simultaneously phosphorylated at Serine-536 (42). IκB-α is constitutively phosphorylated in vivo by casein kinase II (CK2) at serine-293, which alters its intrinsic stability, plays a role in its constitutive turnover and mediates rapid degradation of free IκBα (48–54). RelA is also phosphorylated at serine-529 by CK2 (55).

It is well-established that one of the first genes to be transcriptionally transactivated by NFκB is its inhibitor IκBα (13, 14, 32, 34). This induction functions as a negative feedback mechanism to down-regulate NFκB activity (13, 14, 32, 34). We found that expression of the inhibitory protein IκB-α is elevated in naevus and melanoma biopsies compared with normal skin melanocytes. We postulate that this may reflect enhanced NFκB transcriptional activity in both naevi and melanomas. However, we also found that IκB-α expression was significantly reduced in melanoma relative to naevus biopsies.

These results suggest that increased expression and phosphorylation of RelA occur at the stage of the benign intradermal naevus, but that there may be sufficient levels of the inhibitory protein IκB-α to sequester RelA in the cytoplasm. In contrast, the melanomas also have high RelA expression and high activation to the phosphoserine-529 form of RelA, but have reduced IκB-α expression. The increased nuclear levels of phosphoserine-529 RelA in metastatic melanomas is biologically consistent with both the observed loss in IκB-α expression and the increase in expression of RelA with the unmasked NLS.

NFκB activation has also been reported to transactivate cRel (8). In the current study, we found that expression of cRel is also elevated in melanoma cells of metastatic biopsies in comparison with melanocytes of both naevus and skin biopsies. However, the cRel was retained in the cytoplasmic compartment in the melanomas. In addition to decreased IκB-α expression in melanomas, we also found significantly elevated IκB-ε expression in melanoma compared to naevus biopsies. IκB-ε primarily regulates cRel nuclear translocation (8, 13, 16) and its increased expression in melanomas may explain in part, the predominantly cytoplasmic pattern of cRel expression in melanomas. We also found an equivalent increase in IκB-β expression in melanocytic cells of both naevus and metastatic melanoma biopsies.

In sharp contrast to IκB-α, the inhibitory activity of IκB-β is enhanced by phosphorylation of its C-terminal PEST domain. Basal phosphorylation at consensus CK2 sites of

IκB-β converts it to a strong inhibitor of DNA binding and is required for the efficient formation of IκB-β-cRel complexes (56–58). This may also contribute to the cytoplasmic retention of cRel in melanomas. Therefore we propose that in addition to other kinases such as IKK and Akt, CK2 may also play a contributory role in deregulation of NFκB activity in some melanomas.

Over-expression of RelA may be an early transitional event between melanocytes at the dermal–epidermal junction and those forming a benign intradermal naevus. Under normal circumstances, NFκB nuclear localization is regulated by IκB-α. Signaling mediated by RelA can also be balanced by activation of cRel containing dimers. Generally RelA promotes anti-apoptotic and cRel promotes pro-apoptotic signals. In melanomas however, loss of IκB-α, accumulation of nuclear RelA and absence of nuclear cRel suggest that the NFκB balance in some melanomas may be tipped in favor of constitutive NFκB signaling mediated by RelA. While other pathways including INK4a/ARF (2, 59), PKC (43, 60), PKA (19, 61), Ras (62) and FGF (63, 64) may be involved in the transition to a malignant melanoma, RelA signaling could establish a corps of anti-apoptotic proteins that protect melanomas from death signals as well as radiation and chemotherapy. Recent advances in the development of drugs that down-regulate NFκB signaling may therefore provide potential adjuvants to conventional therapies in some melanomas.

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REFERENCES

1. Margolin KA, Liu PY, Flaherty LE, Sosman JA, Walker MJ, Smith JW, Fletcher WS, Weiss GR, Unger JM, Sondak VK. Phase II study of carmustine, dacarbazine, cisplatin, and tamoxifen in advanced melanoma: A Southwest Oncology Group Study. J Clin Oncol 1998;16:664–669
2. Chin L, Merlino G, DePinho RA. Malignant melanoma: modern black plague and genetic black box. Genes Dev 1998;12: 3467–3481
3. Tang L, Tron VA, Reed JC, Mah KJ, Krajewska M, Li G, Zhou X, Ho VC, Trotter MJ. Expression of apoptosis regulators in cutaneous malignant melanoma. Clin Cancer Res 1998;4:1865–1867
4. McNulty SE, Tohidian NB, Meyskens FL. RelA, p50 and inhibitor of kappa B alpha are elevated in human metastatic melanoma cells and respond aberrantly to ultraviolet light B. Pigment Cell Res 2001;14:456–465
5. Meyskens FL Jr, Buckmeier JA, McNulty SE, Tohidian NB. Activation of nuclear factor-κB in human metastatic melanoma cells and the effect of oxidative stress. Clin Cancer Res 1999;5:1197–1202
6. Devalaraja MN, Wang DZ, Ballard DW, Richmond A. Elevated Constitutive IκB Kinase Activity and IκB-α Phosphorylation in Hs294T Melanoma Cells Lead to Increased Basal MGSA/GRO-α Transcription. Cancer Res 1999;59:1372–1377
7. Yang J, Richmond A. Constitutive IkB kinase activity correlates with nuclear factor-kB activation in human melanoma cells. Cancer Res 2001;61:4901–4909
8. Verma IM, Stevenson JK, Schwarz EM, Van Antwerp D, Miyamoto S. Rel/NF-kappaB/I kappa B family: intimate tales of association and dissociation. Genes Dev 1995;9:2723–2735
9. Chen FE, Ghosh G. Regulation of DNA binding by Rel/NF-kappaB transcription factors: structural views. Oncogene 2003;18:6845–6852
10. Solan NJ, Miyoshi H, Carmona EM, Bren GD, Paya CV. RelB Cellular Regulation and Transcriptional Activity are Regulated by p100. J Biol Chem 2002;277:1405
11. Chen YQ, Ghosh S, Ghosh G. A novel DNA recognition mode by the NF-kappa B p65 homodimer. Nat Struct Biol 1998;5:67–73
12. Karin M, Lin A. NF-κB at the crossroads of life and death. Nat Immunol 2002;3:221–227
13. Li Q, Verma I. NF-kB regulation in the immune system. Nat Rev Immunol 2002;2:725–734
14. Baeuerle PA, Baltimore D. NF-kappaB: ten years after. Cell 1996;87:13–20
15. Chen X, Kandasamy K, Srivastava RK. Differential roles of RelA (p65) and c-Rel subunits of nuclear factor κB in tumor necrosis factor-related apoptosis-inducing ligand signaling. Cancer Res 2003;63:1059–1066
16. Whiteside ST, Epinat JC, Rice NR, Israel A. I kappa B epsilon, a novel member of the I kappa B family, controls RelA and cRel NF-kappa B activity. EMBO J 1997;16:1413–1426
17. Zhong H, May MJ, Jimi E, Ghosh S. The phosphorylation status of nuclear NF-kappa B determines its association with CBP/p300 or HDAC-1. Mol Cell 2002;9:625–636
18. Karin M, Ben Neriah Y. Phosphorylation meets ubiquitination: the control of NF-κB activity. Annu Rev Immunol 2000;18:621–663
19. Zhong H, Voll RE, Ghosh S. Phosphorylation of NF-kappa B p65 by PKA stimulates transcriptional activity by promoting a novel bivalent interaction with the coactivator CBP/p300. Mol Cell 1998;5:661–671
20. Thompson JE, Phillips RJ, Erdjument-Bromage H, Tempst P, Ghosh S. I kappa B-beta regulates the persistent response in a biphasic activation of NF-kappa B. Cell 1995;80:573–582
21. Hirano F, Chung M, Tanaka H, Maruyama N, Makino I, Moore DD, Scheidereit C. Alternative splicing variants of I kappa B beta establish differential NF-kappa B signal responsiveness in human cells. Mol Cell Biol 1998;18:2596–2607
22. Simeonidis S, Stauber D, Chen G, Hendrickson WA, Thanos D. Mechanisms by which I kappa B proteins control NF-kappa B activity. PNAS 1999;96:49–54
23. Chen LF, Fischle W, Verdin E, Greene WC. Duration of nuclear NF-kappa B action regulated by reversible acetylation. Science 2001;293:1653–1657
24. Auphan N, DiDonato JA, Rosette C, Helmberg A, Karin M. Immunosuppression by glucocorticoids: inhibition of NF-kappa B activity through induction of I kappa B synthesis. Science 1995;270:286–290
25. Scheinman RI, Cogswell PC, Lofquist AK, Baldwin AS. Jr Role of transcriptional activation of I kappa B alpha in mediation of immunosuppression by glucocorticoids. Science 1995;270:283–286
26. Jiang HY, Petrovas C, Sonenshein GE. RelB-p50 NF-κB complexes are selectively induced by cytomegalovirus immediate-early protein 1: differential regulation of Bcl-xL promoter activity by NF-κB family members. J Virol 2002;76:5737–5747
27. Shattuck-Brandt RL, Richmond A. Enhanced degradation of I-κBα contributes to endogenous activation of NF-κB in Hs294T melanoma cells. Cancer Res 1997;57:3032–3039
28. Huang S, DeGuzman A, Bucana CD, Fidler IJ. Nuclear factor-κB activity correlates with growth, angiogenesis, and metastasis of human melanoma cells in nude mice. Clin Cancer Res 2000;6:2573–2581
29. Orchard GE. Heavily pigmented melanocytic neoplasms: comparison of two melanin-bleaching techniques and subsequent immunohistochemical staining. Br J Biomed Sci 1999;56:188–193
30. Clark WH Jr, Watson MC, Watcon BEM. Two kind of "clear" cells in the human epidermis. Am J Pathol 1961;39:333
31. Vongtama R, Safa A, Gallardo D, Calcaterra T, Juillard G. Efficacy of radiation therapy in the local control of desmoplastic malignant melanoma. Head Neck – J Sci Spec Head Neck 2003;25:423–428
32. Baichwal VR, Baeuerle PA. Activate NF-kappa B or die? Curr Biol 1997;7:R94–R96
33. Chu ZL, McKinsey TA, Liu L, Gentry JJ, Malim MH, Ballard DW. Suppression of tumor necrosis factor-induced cell death by inhibitor of apoptosis c-IAP2 is under NF-kappa B control. PNAS 1997;94:10057–10062
34. Franco AV, Zhang XD, Van Berkel E, Sanders JE, Zhang XY, Thomas WD, Nguyen T, Hersey P. The role of NF-κB in TNF-related apoptosis-inducing ligand (TRAIL)-induced apoptosis of melanoma cells. J Immunol 2001;166:5337–5345

35. Mayo MW, Wang CY, Cogswell PC, Rogers-Graham KS, Lowe SW, Der CJ, Baldwin AS, Jr. Requirement of NF-'kappa' B Activation to Suppress p53-Independent Apoptosis Induced by Oncogenic Ras. Science 1997;278:1812–1815
36. Wang CY, Mayo MW, Baldwin J. TNF- and cancer therapy-induced apoptosis: potentiation by inhibition of NF-kappa B. Science 1996;274:784–787
37. Dhawan P, Singh AB, Ellis DL, Richmond A. Constitutive activation of Akt/Protein kinase B in melanoma leads to up-regulation of nuclear factor-κB and tumor progression. Cancer Res 2002;62:7335–7342
38. Meng FY, Liu L, Chin PC, D'Mello SR. Akt is a downstream target of NF-kappa B. J Biol Chem 2002;277:29674–29680
39. Sizemore N., Leung S., Stark GR 1. Activation of phosphatidylinositol 3-kinase in response to interleukin-1 leads to phosphorylation and activation of the NF-B p65/RelA subunit. Mol Cell Biol 1999;19:4798–4805
40. Madrid LV, Wang CY, Guttridge DC, Schottelius AJ, Baldwin AS Jr, Mayo MW. Akt suppresses apoptosis by stimulating the transactivation potential of the RelA/p65 subunit of NF-κB. Mol Cell Biol 2000;20:1626–1638
41. Meng F, D'Mello SR. NF-kappa B stimulates Akt phosphorylation and gene expression by distinct signaling mechanisms. Biochim Biophys Acta 2003;1630:35–40
42. Vermuelen L, DeWilde G, Notebaert S, Vanden Berghe W, Haegeman G. Regulation of the transcriptional activity of the nuclear factor-κB p65 subunit. Biochem Pharmacol 2002;64:963–970
43. Duran A, Diaz-Meco MT, Moscat J. Essential role of RelA Ser311 phosphorylation by zeta PKC in NF-kappa B transcriptional activation. Embo J 2003;22:3910–3918
44. Mayo MW, Denlinger CE, Broad RM, Yeung F, Reilly ET, Shi Y, Jones DR. Ineffectiveness of histone deacetylase inhibitors to induce apoptosis involves the transcriptional activation of NF-κB through the Akt pathway. J Biol Chem 2003;278:18980–18989
45. Madrid LV, Mayo MW, Reuther JY, Baldwin AS. Akt stimulates the transactivation potential of the RelA/p65 subunit of NF-kappa B through utilization of the I kappa B kinase and activation of the mitogen-activated protein kinase p38. J Biol Chem 2001;276:18934–18940
46. DelHause M, Li N, Karin M. Signalling pathways: kinase regulation in inflammatory response. Nature 2000;406:368
47. Verma IM, Stevenson J. Ikappa B kinase: beginning, not the end. PNAS 1997;94:11758–11760
48. Pando MP, Verma IM. Signal-dependent and -independent degradation of free and NF-kappa B-bound I kappa B alpha. J Biol Chem 2000;275:21278–21286
49. Barroga CF, Stevenson JK, Schwarz EM, Verma IM. Constitutive phosphorylation of IκBα by casein kinase II. PNAS 1995;92:7637–7641
50. Lin R, Beauparlant P, Makris C, Meloche S, Hiscott J. Phosphorylation of I kappa B alpha in the C-terminal PEST domain by casein kinase II affects intrinsic protein stability. Mol Cell Biol 1996;16:1401–1409
51. Schwarz EM, Van Antwerp D, Verma IM. Constitutive phosphorylation of I kappa B alpha by casein kinase II occurs preferentially at serine 293: requirement for degradation of free I kappa B alpha. Mol Cell Biol 1996;16:3554–3559
52. Wang D, Westerheide SD, Hanson JL, Baldwin AS Jr. Tumor necrosis factor alpha – induced phosphorylation of RelA/p65 on Ser529 is controlled by casein kinase II. J Biol Chem 2000;275:32592–32597
53. Bird TA, Schooley K, Dower SK, Hagen H, Virca GD. Activation of nuclear transcription factor NF-kappa B by interleukin-1 is accompanied by casein kinase II-mediated phosphorylation of the p65 subunit. J Biol Chem 1997;272:32606–32612
54. Ravi R, Bedi A. Sensitization of tumor cells to Apo2 ligand/TRAIL-induced apoptosis by inhibition of casein kinase II. Cancer Res 2002;62:4180–4185
55. Wang D, Westerheide SD, Hanson JL, Baldwin AS. Tumor necrosis factor alpha-induced phosphorylation of RelA/p65 on Ser(529) is controlled by casein kinase II. J Biol Chem 2000;275:32592–32597
56. Good L, Sun SC. Persistent activation of NF-kappa B/Rel by human T-cell leukemia virus type 1 tax involves degradation of I kappa B beta. J Virol 1996;70:2730–2735
57. Tran K, Merika M, Thanos D. Distinct functional properties of I kappa B alpha and I kappa B beta. Mol Cell Biol 1997;17:5386–5399
58. Chu ZL, McKinsey TA, Liu L, Qi X, Ballard DW. Basal phosphorylation of the PEST domain in the I(kappa) B (beta) regulates its functional interaction with the c-rel proto-oncogene product. Mol Cell Biol 1996;16:5974–5984
59. Sharpless E, Chin L. The INK4a/ARF locus and melanoma. Oncogene 2003;22:3092–3098
60. Gilhooly EM, Morse-Gaudio M, Bianchi L, Reinhart L, Rose DP, Connolly JM, Reed JA, Albino AP. Loss of expression of protein kinase C beta is a common phenomenon in human malignant melanoma: a result of transformation or differentiation? Melanoma Res 2001;11:355–369
61. Becker D, Beebe SJ, Herlyn M. Differential expression of protein kinase C and cAMP-dependent protein kinase in normal human melanocytes and malignant melanomas. Oncogene 1990;5:1133–1139
62. Bardeesy N, Bastian BC, Hezel A, Pinkel D, DePinho RA, Chin L. Dual inactivation of RB and p53 pathways in RAS-induced melanomas. Mol Cell Biol 2001;21:2144–2153
63. Meier F, Caroli U, Satyamoorthy K, Schittek B, Bauer J, Berking C, Moller H, Maczey E, Rassner G, Herlyn M, Garbe C. Fibroblast growth factor-2 but not Mel-CAM and/or beta3 integrin promotes progression of melanocytes to melanoma. Exp Dermatol 2003;12:296–306
64. Yayon A, Ma YS, Safran M, Klagsbrun M, Halaban R. Suppression of autocrine cell proliferation and tumorigenesis of human melanoma cells and fibroblast growth factor transformed fibroblasts by a kinase-deficient FGF receptor 1: evidence for the involvement of Src-family kinases. Oncogene 1997;14:2999–3009