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# Role of the Brn-3a and Brn-3b POU family transcription factors in cancer

**Review Article**

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**Abbreviations:** cervical intra-epithelial neoplasia Type 3, (CIN3)

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### Summary

**Brn-3a and Brn-3b are closely-related POU family transcription factors both of which play an important role in the nervous system. However, both these factors were originally isolated from a neuroblastoma cell line and their expression has been shown to be altered in several different human cancers. Interestingly, functional studies have shown that Brn-3b has a growth-stimulating effect in neurobastomas, whereas Brn-3a has a growth-inhibiting effect. Similarly, Brn-3b is over-expressed in human breast cancers and stimulates their growth. However, Brn-3a is strongly over-expressed in human cervical cancer and stimulates cervical tumour growth by activating expression of the human papilloma virus E6 and E7 oncogenes which are essential for development of this tumour. Hence, these closely-related factors play critical but distinct roles in different human cancers.**

## I. Introduction

The POU family of transcription factors was originally defined on the basis of a common DNA binding domain identified in the mammalian transcription factors Pit, Oct-1 and Oct-2 and the nematode regulatory protein Unc-86 (Herr et al, 1988). Subsequently, a large number of other POU family members have been identified in a range of different invertebrates and vertebrates and have been shown to play critical roles in development, particularly in the nervous system (Verrijzer and van der Vliet 1993; Ryan and Rosenfeld 1997; Latchman 1999).

For example, He et al, (1989) used degenerate oligonucleotides corresponding to conserved regions of the POU domain to isolate several novel POU factors expressed specifically in the brain. One of these, which they named Brn-3 was highly expressed in sensory neurones of the peripheral nervous system and was particularly closely related to the nematode Unc-86 gene product, indicating the evolutionary conservation of POU proteins.

Subsequently however, using a similar approach in a rodent neuroblastoma cell line we isolated two very closely-related POU factors (Lillycrop et al, 1992). One of these was identical to the Brn-3 factor reported by He et al, (1989), whilst the other showed seven amino acid differences in the POU domain from the original factor. Subsequent studies indicated that these two factors which we named respectively Brn-3a and Brn-3b, were encoded by different genes and, whilst having highly homologous POU domains, were much less homologous outside the POU domain (Lillycrop et al, 1992: Ring and Latchman, 1993). Subsequently, a third closely-related factor Brn-3c was also isolated from the nervous system (Ninkina et al, 1993).

All these three factors play essential roles in development of particular aspects of the nervous system. Thus, inactivation of Brn-3a (also known as Brn-3.0) in knock out mice results in extensive death of sensory neurones and is incompatible with survival (McEvilly et al, 1996; Xiang et al, 1996). Although inactivation of Brn-3b (also known as Brn-3.2) and Brn-3c (also known as Brn-3.1) is not incompatible with survival of the animal, such inactivation leads respectively to defects in the visual and auditory systems (Erkman et al, 1996; Xiang et al, 1997). Hence, the POU factors Brn-3a, Brn-3b and Brn-3c constitute a closely-related group of factors which are classified together in the POU IV subfamily and are the most closely-related mammalian factors to Unc-86 and like this factor play an essential role in the proper development of the nervous system.

However, in terms of cancer it is of particular interest that both Brn-3a and Brn-3b were isolated from a rodent

neuroblastoma cell line and were shown to be regulated during its differentiation (Lillycrop et al, 1992). Similarly, Brn-3a was also isolated independently (and named RDC-1) as a factor which is expressed by Ewing's sarcomas (Collum et al, 1992) and was subsequently shown to be expressed in a number of aggressive neuroendocrine tumours (Leblond-Francillard et al, 1997). Similarly, Brn-3b was shown to be expressed by teratocarcinoma cell lines and to be regulated during their differentiation (Turner et al, 1994). These early expression studies led to the suggestion that these factors may play a particularly critical role in specific cancers (Chiarugi et al, 2002). In this review, I will discuss detailed studies on a few tumour cell types which indicate that this is indeed the case and which demonstrate critical but contrasting roles for Brn-3a and Brn-3b in different types of cancer.

## II. Brn-3a and Brn-3b in neuroblastoma

As indicated above, Brn-3a and Brn-3b were originally isolated from a rodent neuroblastoma cell line (Lillycrop et al, 1992). When these cells are induced to differentiate from a dividing cell type to a non-dividing cell bearing numerous neurite processes, the level of Brn-3a was shown to increase dramatically, whilst the level of Brn-3b decreased (Lillycrop et al, 1992; Budhram-Mahadeo et al, 1994, 1995). A similar increase in Brn-3a and decrease in Brn-3b was also noted when several different human neuroblastoma cell lines were induced to differentiate in culture (Smith and Latchman, 1996).

These expression studies were of particular interest since Brn-3a and Brn-3b were shown to have antagonistic effects on their target promoters. Thus, Brn-3a was able to activate the promoters of genes encoding neuronal differentiation markers such as SNAP-25 and the neurofilaments, whereas Brn-3b repressed these promoters and antagonised their activation by Brn-3a (Lakin et al, 1995; Smith et al, 1997c). This led to the idea that Brn-3a may act to promote neuroblastoma differentiation by inducing the activity of neuronal differentiation genes, whilst Brn-3b opposes such an effect and promotes the maintenance of the non-differentiated proliferative phenotype.

This idea was directly proven by over-expressing Brn-3a in neuroblastoma cells in the absence of a differentiation stimulus. This resulted in the cells activating neuronal specific genes and undergoing differentiation to a process-bearing cell type (Smith et al, 1997b). Conversely, over-expression of Brn-3b in these cells prevented neuronal differentiation even in response to stimuli which would normally induce it (Smith et al, 1997a). Hence, Brn-3a can indeed promote the differentiation of neuroblastoma cells whereas Brn-3b opposes this effect and promotes their continued proliferation.

Interestingly, the ability of full length Brn-3a to activate neuronal-specific genes and induce differentiation can be produced by the isolated POU domain, whereas such effects are not observed with the POU domain of Brn-3b which differs by only seven amino acids (Smith et al, 1997b). The critical difference between Brn-3a and Brn-3b resides at position 22 in the POU-homeodomain (which is one of the two subdomains of the POU domain). Thus, altering the valine found at this position in Brn-3a to the isoleucine found in Brn-3b abolishes its ability to activate neuronal-specific gene expression and induce differentiation, whereas the converse change introducing a valine into Brn-3b allows it to activate neuronal-specific gene expression and induce differentiation, even though only a single amino acid has been changed (Dawson et al, 1996; Smith et al, 1997b).

These studies indicate that a small difference between Brn-3a and Brn-3b allows Brn-3a to act as an inducer of differentiation in neuroblastoma cells, whereas Brn-3b opposes this effect.

Although these findings were based on *in vitro* studies of a rodent cell line, they have recently been extended to a human neuroblastoma cell line using both *in vitro* and *in vivo* techniques. Thus, overexpression of Brn-3b in a human neuroblastoma cell line results in its enhanced proliferation, whereas inhibition of Brn-3b expression correspondingly leads to reduced proliferation. Interestingly, overexpression of Brn-3b also results in the increased ability of these human neuroblastoma cells to show anchorage-independent growth in culture, as well as demonstrating increased invasiveness based on their ability to migrate through an artificial matrigel basement membrane (Irshad et al, 2004). Most importantly, these studies were also extended to the *in vivo* situation by showing that the human neuroblastoma cells with enhanced Brn-3b showed an increased ability to form tumours when introduced into nude mice compared to control cells, whereas the cells with reduced Brn-3b showed a decreased ability to form tumours (Irshad et al, 2004). These results therefore, extend the initial findings and indicate that Brn-3b appears to be a potent enhancer of tumour growth and invasiveness

## III. Brn-3a and Ewing's sarcoma

As noted above, Collum et al, (1992), observed expression of Brn-3a (which they referred to as RDC-1) in Ewing's sarcoma/primitive neuroectodermal tumour cells, which like neuroblastomas are tumours derived from the neuroendocrine lineage of neural crest cells (Kovar 1998; da Alva and Gerald, 2000). These tumours are characterised by rearrangement of the gene encoding the EWS regulatory protein to form a fusion protein with a member of the Ets family of transcription factors with the resulting fusion protein acting as a strong transcriptional regulator, which unlike either parental factor can produce cellular transformation. In 85% of cases, the gene rearrangement involves the production of a fusion protein containing the N-terminal part of EWS linked to the C-terminal portion of the Ets family transcription factor Fli-1 (Arvand and Denny, 2001; Ladanyi, 2002).

In view of the expression of Brn-3a in these tumours, it is of particular interest that in a yeast two hybrid screen for proteins which interact with Brn-3a, we isolated the EWS protein and subsequently showed that Brn-3a can

interact with both EWS and its oncogenic derivative EWS-Fli1 (Thomas and Latchman, 2002).

Most interestingly however, the interaction between Brn-3a and EWS or EWS-Fli1 has different functional effects. Thus, interaction of Brn-3a with EWS-Fli1 prevents Brn-3a from activating markers of neuronal differentiation and inducing neurite outgrowth and also inhibits its ability to activate the promoter of the p21 cell cycle arrest gene and to induce cell cycle arrest (Gascoyne et al, 2004) (**Figure 1**).

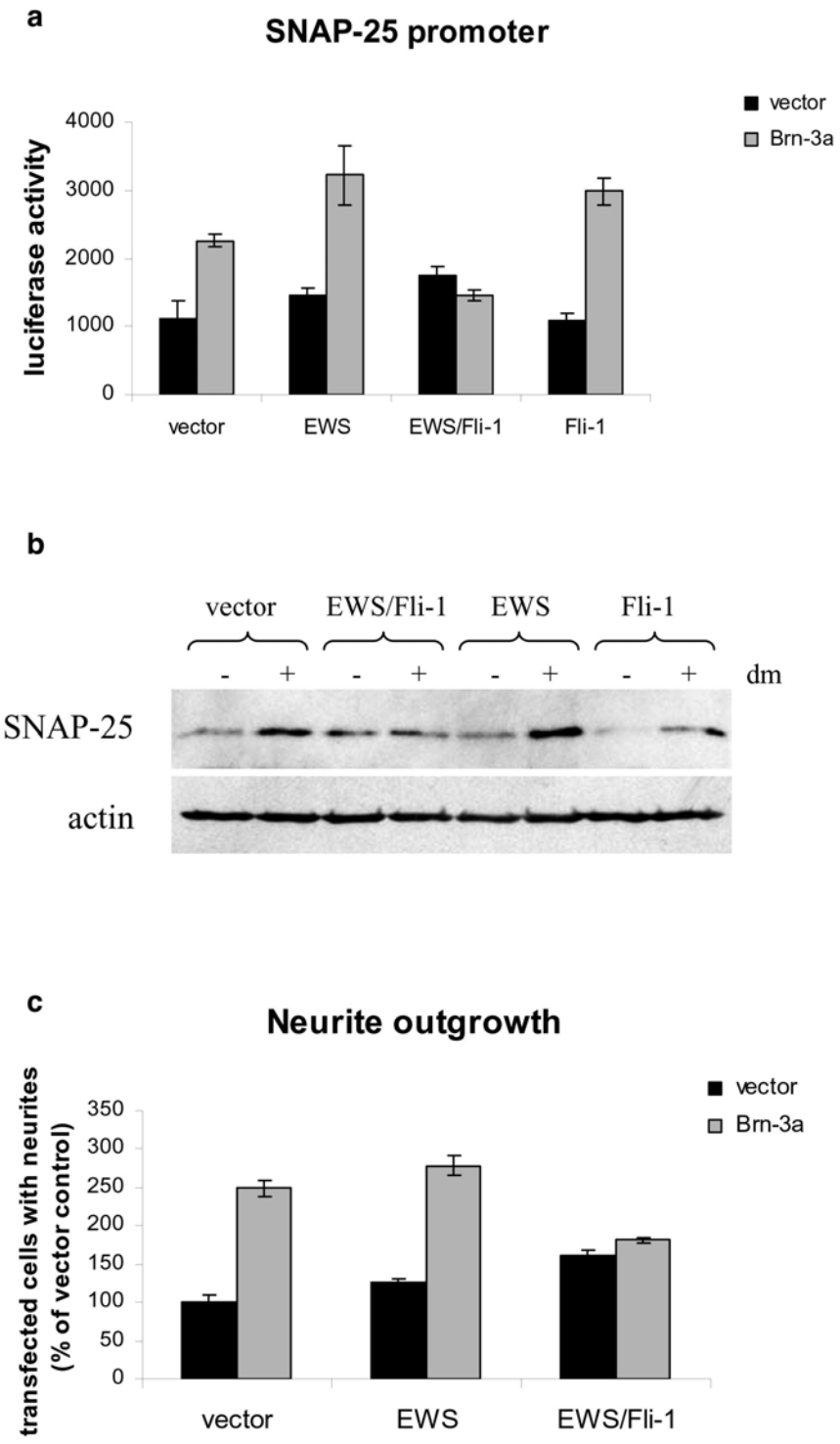


**Figure 1**. Effect of EWS or EWS/Fli1 on the ability of Brn-3a to induce the SNAP-25 promoter (**panel a**), the endogenous SNAP-25 gene (**panel b**) and neurite outgrowth (**panel c**). Note the manner in which EWS/Fli1 but not EWS blocks the effect of Brn-3a. In panels a and c, Brn-3a was introduced by transfection, in panel b endogenous Brn-3a expression was induced with differentiation medium.

In contrast, interaction with EWS does not inhibit these effects of Brn-3a. Hence, the rearrangement which results in the production of EWS-Fli1 produces a protein which is able to inhibit the growth arrest and differentiation-inducing properties of Brn-3a, thereby promoting tumour cell growth.

Interestingly, Brn-3a in addition to its effect on differentiation can also activate genes encoding anti-apoptotic proteins such as Bcl-2 and Bcl-x and correspondingly protect neuronal cells from apoptosis (Smith et al, 1998b; Ensor et al, 2001). These effects, unlike the effects on neuronal differentiation require an additional N-terminal domain of Brn-3a (Smith et al, 1998a; 2001). Clearly, this anti-apoptotic effect of Brn-3a has the potential to promote tumour cell survival and may therefore be antagonistic to the effect inducing tumour cell differentiation. Indeed, in an early study, Thiel et al, (1993), reported that Brn-3a could co-operate with the Ras oncogene to induce oncogenic transformation and that this effect was dependent upon the presence of the N-terminal domain.

In this regard, it is therefore of particular interest that the interaction of Brn-3a with EWS and EWS-Fli1 appears to affect the anti-apoptotic activity of Brn-3a differently compared to its differentiation/growth arrest effect. Thus, EWS but not EWS-Fli1 can prevent the activation of the Bcl-2 and Bcl-x promoters by Brn-3a and inhibit its anti-apoptotic effect (Thomas and Latchman, 2002; Gascoyne et al, 2004).

Hence, the oncogenic rearrangement of EWS to produce EWS-Fli1 releases the EWS-mediated block on the anti-apoptotic effect of Brn-3a, thereby promoting tumour cell survival, whilst simultaneously inhibiting its growth arrest/differentiation-inducing effect, thereby promoting tumour growth.

## IV. Brn-3b in breast cancer

Although Brn-3b can also interact with EWS and EWS-Fli1, this interaction is much weaker than that with Brn-3a and its functional significance in Ewing's sarcoma is at present unclear (Gascoyne et al, 2004). Interestingly however, a role for Brn-3b in breast cancer has been defined and appears to be similar to that described above for neuroblastoma.

Thus, human MCF-7 breast cancer cells which have been engineered to overexpress Brn-3b, exhibit enhanced proliferation and anchorage-independent growth, whereas cells engineered to have reduced Brn-3b levels show reduced growth and anchorage independence (Dennis et al, 2001). Moreover, overexpression of Brn-3b in MCF-7 cells enhances their responsiveness to oestrogen which is correspondingly reduced in the cells showing reduced Brn-3b levels. This is in agreement with previous molecular analysis which showed that Brn-3b can interact directly with the oestrogen receptor via a protein-protein interaction, which results in enhanced transcriptional activity of the receptor (Budhram-Mahadeo et al, 1998).

These effects on a human breast cancer cell line in culture are of particular interest since Brn-3b has also been shown to be overexpressed in human mammary tumour biopsies compared to its level in normal human mammary gland tissue (Budhram-Mahadeo et al, 1999). In contrast, no overexpression of Brn-3a was observed. Moreover, expression of Brn-3b in the human breast cancer biopsies correlates inversely with the expression of the BRCA-1 anti-oncogene. This suggests that Brn-3b may repress expression of the BRCA-1 anti-oncogene in sporadic cancers, producing the same effect as the mutation of this anti-oncogene which occurs in inherited breast cancer. In agreement with this idea, Brn-3b can repress the BRCA-1 promoter in co-transfection experiments (Dennis et al, 2001).

To further probe the way in which Brn-3b can alter breast cancer cell growth, we also carried out a transcriptomic/gene chip screen to identify novel genes whose expression was altered in Brn-3b overexpressing breast cancer cells compared to cells with reduced expression. This resulted in the identification of a number of different genes whose expression is either increased or decreased in breast cancer cells, when Brn-3b expression is altered (Samady et al, 2004) (**Table 1**). Most interestingly, one of these encodes the cyclin-dependent kinase 4 (CDK4) which plays a critical role in stimulating cellular growth. Following the initial identification of CDK4 as a putative target gene for Brn-3b, we were able to demonstrate that expression of CDK4 correlates positively with Brn-3b expression in breast cancer biopsy material and that Brn-3b can activate the CDK4 promoter (Samady et al, 2004)

As well as demonstrating that Brn-3b is likely to play a stimulatory role in breast cancer as well as in neuroblastoma, these experiments demonstrate the variety of mechanisms by which Brn-3b may act to achieve this effect. Thus, it appears that Brn-3b can repress the expression of the anti-oncogenic protein BRCA-1, whilst stimulating the transcription of the gene encoding the growth-promoting CDK4 protein and interacting with the oestrogen receptor to stimulate its transcriptional activating ability.

## V. Brn-3a in cervical cancer

The studies described so far, have indicated a strong stimulatory role for Brn-3b in both breast cancer and neuroblastoma. Conversely, Brn-3a expression is unchanged in breast cancer and appears to have a predominantly anti-oncogenic role in both neuroblastoma and Ewing's sarcoma.

At first sight therefore, it is perhaps surprising that human cervical biopsies demonstrate a 300-fold elevation in Brn-3a expression in cervical intra-epithelial neoplasia Type 3 (CIN3) compared to normal biopsies from women with a normal cervix (Ndisang et al, 1998). In contrast, no difference is observed between Brn-3b levels in CIN3 and normal cervix. This paradox is explained by the fact that Brn-3a but not Brn-3b can activate the upstream regulatory region of human papilloma viruses Types 16 and 18 (HPV-16 and HPV-18), which controls the production of the oncogenic E6 and E7 proteins (Morris et al, 1994).

In agreement with the idea that Brn-3a acts in cervical cells via stimulating HPV oncogene expression, overexpression of Brn-3a in cervical cell lines containing

HPV enhances their expression of HPV E6 protein, stimulates their cellular growth and their ability to grow in an anchorage-independent manner, whereas none of these effects are observed when Brn-3a is over-expressed in cervical cell lines which do not contain HPV genomes(Ndisang et al, 1999). Most importantly, cervical cells engineered to have reduced levels of Brn-3a not only exhibit reduced E6 expression and cellular growth in culture, but also show a decreased ability to form tumours in nude mice, demonstrating that Brn-3a is important for tumour growth *in vivo* (Ndisang et al, 2001).

**Table 1.** Genes showing altered expression in MCF-7 cells over expressing Brn-3b compared to those with reduced levels of Brn-3b

| **Ratio** | | |
|---|---|---|
| **Up** | **Down** | **Gene** |
| | 2.4 | c-jun proto-oncogene; transcription factor AP-1 |
| 8.3 | | Interferon-inducible protein 9-27 |
| | 2.0 | c-myc oncogene |
| 2.1 | | c-myc binding protein MM-1 |
| 1.7 | | cell division protein kinase 4; cyclin-dependent kinase 4 (CDK4); PSK-J3 |
| 2.3 | | cyclin-dependent kinase inhibitor 1 (CDKN1A); melanoma differentiation-associated protein 6 (MDA6); CDK-interacting protein 1 (CIP1); WAF1 |
| 1.7 | | cyclin-dependent kinase regulatory subunit 1 (CKS1) |
| | 1.8 | cdc2-related protein kinase PISSLRE |
| | 3.5 | G1 to S phase transition protein 1 homologue; GTP-binding protein GST1-HS |
| | 1.7 | ADP/ATP carrier protein |
| | 2.1 | protein phosphatase 2C gamma |
| 1.9 | | rhoC (H9); small GTPase (rhoC) |
| 1.7 | | B-cell receptor-associated protein (hBAP) |
| | 13.7 | zyxin + zyxin-2 |
| 1.7 | | c-jun N-terminal kinase 2 (JNK2); JNK55 |
| | 4.5 | junction plakoglobin (JUP); desmoplakin III (DP3) |
| | 2.4 | DNA ligase 1; polydeoxyribonucleotide synthase (ATP) (DNL1) (LIG1) |
| 1.9 | | tumour necrosis factor type 1 receptor associated protein (TRAP1) |
| | 8.0 | TIS11B protein, EGF response factor (ERF1) |
| | 5.6 | early growth response protein 1 (hEGR1); transcription factor ETR103; KROX24; zinc finger protein 225; AT225 |
| | 8.3 | fuse-binding protein 2 (FBP2) |
| | 2.0 | transcription factor erf-1; AP2 gamma transcription factor |
| | 1.9 | integrin beta 4 (ITG84); CD104 antigen |
| | 2.1 | high mobility group protein HMG2 |
| | 3.4 | paxillin |
| | 2.3 | alpha 1 catenin (CTNNA1); cadherin-associated protein; alpha E-catenin |
| 2.0 | | glutathione-S-transferase (GST) homologue |
| | 5.5 | 78-kDa glucose regulated protein precursor (GRP 78); immunoglobulin heavy chain binding protein (BIP) |
| | 2.0 | cathepsin D precursor (CTSD) |
| | 2.0 | interleukin-1 beta precursor (IL-1; IL1B); catabolin |
| 1.8 | | macrophage migration inhibitory factor (MIF); glycosylation-inhibiting factor (GIF) |
| | 2.1 | 60S ribosomal protein L5 |
| 2.6 | | ornithine decarboxylase |
| | 3.9 | PM5 protein |
| | 1.7 | suppressor for yeast mutant |
| | 1.8 | type 11 cycloskeletal 2 epidermal keratin (KRT2E);cytokeratin 2E |
| | 3.5 | (K2E;CK2E) |
| 1.8 | | glycyl tRNA synthetase |
| | | aminoacylase 1 (ACY1) |

**Table 2.** Brn-3a and E-6 levels in Pap smears from patients categorised on the basis of the histological diagnosis

| **Category** | **Count (No =)** | **Percentage** | **Brn-3a mean value** | **E-6 mean value** |
|---|---|---|---|---|
| Negative | 74 | 31% | 0.201 | 0125 |
| LGSIL (HPV-CIN1) | 83 | 35% | 0.259 | 0.231 |
| HGSIL (CIN2-CIN3) | 79 | 33% | 0.438 | 0.358 |
| Cancer | 2 | 1% | 0.575 | 0.475 |
| **Total** | **238** | **100%** | **-** | **-** |

Hence, Brn-3a appears to be of importance as a cellular factor which is required to stimulate HPV gene expression and hence produce oncogenic transformation following initial infection with HPV-16 or HPV-18. Interestingly, Brn-3a levels are also elevated in biopsies from women with CIN3 when the biopsy is taken from a normal region of the cervix (Ndisang et al, 1998; 2000). This suggests therefore, that Brn-3a is not specifically elevated in the tumour cells. Rather, it may be elevated in a subset of women for genetic or environmental reasons and that such women are at enhanced risk of tumour formation following initial infection with HPV. This is of particular importance since the vast majority of women clear HPV infections and do not progress to tumour formation.

Although our initial studies on Brn-3a expression were conducted on cervical biopsies, we have recently been able to measure Brn-3a in routinely taken cervical smear samples (Sindos et al, 2003b). As elevated levels of Brn-3a in the smear correlate with the presence of cervical abnormality as determined by subsequent histological analysis (**Table 2**), its measurement may represent an additional test which could be used to confirm the results of cytological examination and determine the need for further action. Moreover, Brn-3a levels are elevated in cervical smears from women with persistent minor smear abnormalities who were subsequently found by histological examination to have CIN2/3 compared to those with CIN1 or no abnormality (Sindos et al, 2003a). This suggests that Brn-3a could be used as a marker for women who require detailed follow-up in this situation since they would be predicted to be at enhanced risk of disease-progression. Hence, as well as playing a critical role in the development of cervical tumours, Brn-3a may represent a novel prognostic and diagnostic marker of the disease.

## VI. Conclusion

The studies described above have characterised the role of Brn-3a and Brn-3b in several different tumours. They have indicated that Brn-3b plays a stimulatory role in tumours such as neuroblastoma and breast cancer, whilst Brn-3a may have an anti-oncogenic role in neuroblastoma and Ewing's sarcoma but is involved in the development of cervical cancer, via its ability to activate human papilloma virus gene expression.

These findings suggest that it would be worthwhile to investigate the role of Brn-3 factors in other types of tumour. This is particularly so in view of recent findings using gene chip analysis which have suggested that Brn-3a is specifically overexpressed in leukaemias with the t(8;21) translocation (Schoch et al, 2002; Debernardi et al, 2003). Similarly, it is of interest that the gene encoding Brn-3b has recently been shown to be activated by the Wilms' tumour suppressor protein WT-1 (Wagner et al, 2003), whilst Brn-3c has been shown to be overexpressed in Merkel cell carcinoma (Lennard et al, 2002). The characterisation of the role of Brn-3a, Brn-3b and Brn-3c in different types of tumours is likely therefore to require considerably more effort. It is already clear however, in the case of Brn-3a and Brn-3b that both these factors play a critical role in specific types of human cancer where their expression is altered.

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