ANTICANCER RESEARCH *25*: 1075-1078 (2005)

The JNK Interacting Protein JIP-1 and Insulin Like Growth Factor II Genes are Co-expressed in Human Embryonic Tumours

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Abstract. *JNK interacting protein 1 (JIP-1) is a pivotal scaffolding protein in the JNK signalling pathway. It is believed to play a role in the mediation of mitogenic messages from the plasma membrane to the cell interior. Recent evidence suggests that the JIP-1 gene is co-regulated with the insulin like growth factor II (IGF II) gene, thereby contributing to the growth-promoting effects of this potent growth factor. In this study, fourteen embryonic tumours were examined for the expression of JIP-1 and IGF II. It was found that, irrespective of histological type and expression level, the two genes showed a high degree of co-variation in the sense that high IGF II expression was followed by high expression of JIP-1. This finding further supports the notion that JIP-1 and IGF II act in concert to enhance cell proliferation.*

Germ cell tumours, like other malignant neoplasms, are characterised by unrestricted proliferation *in vivo*. Even though there has been an unprecedented breakthrough in the therapeutic management of these tumours, their cell biology is still incompletely understood. Twenty years ago, developmental tumours were often used as an indirect means of studying mammalian embryogenesis. We showed that, like in the early mammalian embryo, the Insulin like Growth Factor II (IGF II) gene was sometimes expressed at high levels in primary testicular tumours of different histological phenotype (1). Since then, a number of studies have confirmed a high degree of IGF II expression in a variety of developmental tumours (2, 3).

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Key Words: IGF II, JIP-1, testicular tumours.

Mitogen Activated Protein Kinases (MAPKs) play a central role in the intracellular mediation of signals for a plethora of cellular events such as proliferation, growth, differentiation, locomotion, survival and oncogenic transformation. Polypeptide growth factors, hormones and cytokines are capable of activating different intracellular MAPK cascades. In addition, gamma irradiation, heat shock and disturbed osmotic balance can elicit a cellular response *via* the MAPK pathway (4, 5).

In the MAPK pathway interest has focussed on a set of key proteins – the c-Jun amino terminal kinases (JNK). These proteins have been shown to play a pivotal role in the control of early embryonic development in *Drosophila* (6). In mammals, JNK proteins play a more diverse role and are nowadays believed to be involved in oncogenic transformation (7-10).

JIP-1 (JNK Interacting Protein 1) was discovered on the basis of its ability to interfere with JNK (11). Initially JIP-1 was described as a cytosolic peptide that binds to JNK to prevent its localisation to the nucleus (11). Subsequently, JIP-1 was found to bind several members of the JNK cascade, suggesting that it acts as a scaffold for the JNK signalling pathway (12). The mechanism of scaffolding and anchoring adds another level of specificity to the intracellular signalling system, since it co-ordinates the binding of specific proteins and consequently elicits a specific response (13). Mutations in the JIP-1 gene result in an inadequate regulation of the JNK transduction and, consequently, give rise to various pathological conditions including certain forms of cancer (11).

Since JIP-1 appears to play an important role in intracellular signal transduction (14, 15), we studied whether the expression of the JIP-1 gene is in any way related to the growth phenotype of tumours that resemble human embryonic tissue. It has been known for some time that IGF II is the most ubiquitous growth factor in the mammalian embryo (2) We have previously shown that IGF II expression and JIP-1 expression are closely linked in the foetal liver, thereby suggesting that JIP-1 acts as a downstream mediator of growth factor action. Since a

Table I. *Histological typing of the testicular tumours under study.*

1.	Seminoma
2.	Teratoma
3.	Seminoma/Embryonal Carcinoma
4.	Embryonal Carcinoma
5.	Embryonal Carcinoma
6.	Embryonal Carcinoma
7.	Embryonal Carcinoma
8.	Yolk Sac Tumour
9.	Embryonal Carcinoma
10.	Embryonal Carcinoma
11.	Leydig Cell Tumour
12.	Seminoma
13.	Seminoma
14.	Seminoma

variety of embryonic tumours overexpress IGF II, which is commonly believed to reflect their high proliferative rate, it became of interest to examine whether this link is maintained even when growth control is relaxed. The data presented in this study suggest that, in most embryonic tumours, the expression of the IGF II and JIP-1 genes is correlated.

Materials and Methods

Primary material. Fourteen human testicular tumours were surgically removed and used for this study. Parts of the tumour were taken for histological classification by light microscopy after staining in Haematoxylin/eosin and/or Giemsa.

RNA extraction and Northern blotting. Total RNA from surgically removed tumours was extracted by a standard Trizol/Chloroform extraction procedure In each case, the quality was checked by running the samples on an ethidium bromide-containing minigel. Moreover, in each case quantification was carried out by spectrophotometry. From these total RNA samples, polyadenylated RNA was purified using an Oligo dT cellulose-based purification technique described by Hyldahl *et al.* (16) One μg of polyadenylated RNA from each tumour (Table I) was run on a denaturing agarose/formaldehyde gel (16). The electrophoresed RNA was then transferred by blotting onto Hybond N+ filter (Amersham Pharmacia Biotech, Sweden), cross-linked by UV light and stored in a sealed plastic bag until further use.

cDNA probes, radioactive labelling and hybridisation. For the analysis of gene expression, two probes were used; a 500 bp mouse IGF II coding sequence cDNA (from Dr A Shokrai, Uppsala, Sweden) and a 2832 bp murine JIP-1 cDNA fragment (11). The cDNAs were labelled with 32P-dCPT by using a Megaprime DNA labelling system (Amersham Life Science). The filters were hybridised in a prefabricated hybridisation buffer supplied by Amersham Pharmacia Biotech, as described by the manufacturer's instructions. After hybridisation, the filters were washed to a stringency level of 0.1 x SSC, 55˚C, air-dried and subjected to autoradiography. To obtain a comparable value of the relative JIP-1 and IGF II expression, each film was subjected to densitometry and the integrated IGF II and JIP-1 values were divided by each other. All values were normalised by multiplication with a constant so that the wild-type relationship in a reference embryonic tissue was given the relative value 1 (17, 18)

Table II. *The relative expression of the IGF II and JIP-1 genes (arbitrary values).Finally the relationship between IGF II and JIP-1-expression was calculated as a quota based on densitometric measurements of X-ray films.*

Tumour no.	IGF II expr.	JIP-1 expr	IGF II/JIP-1
1.	+	(+)	0.8
2.	++	++	1.1
3.	+++	(+)	9.4*
4.	+	+	1.0
5.	++	(+)	3.1*
6.	+	+	0.9
7.	+	++	0.7
8.	+	++	0.7
9.	++	++	1.0
10.	++++	++++	1.2
11.	(+)	(+)	1.3
12	+	(+)	1.6
13.	+	+	1.1
14.	(+)	(+)	1.2

*= Statistically different. $p<0.025$

Statistics. The statistical difference between means was calculated by Student's *t*-test. The level of significance was set at $p=0.05$

Results

Table I shows the histological examination of the fourteen tumours used for this study. Four were identified as seminoma, one as a Leydig cell tumour, one as a yolk sac tumour, one as a teratoma (mature), six embryonal carcinomas and one mixed seminoma/embryonal carcinoma.

From each of the tumours, poly A+ RNA was purified and separated on a gel. The amount of poly A+ RNA loaded onto the gel was quantified by spectrophotometry and checked by running a series of dilutions on a minigel prior to the experiment. To make a valid comparison of the

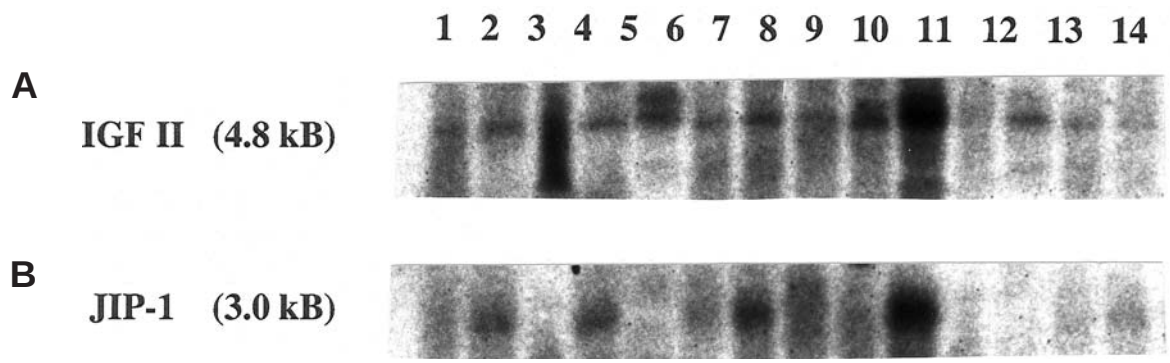

Figure 1. *The expression of IGF II (upper panel) and JIP-1 (lower panel) in fourteen testicular tumours (1-14 as classified in Table I).*

expression pattern of the two genes in the different tumour samples, each filter was first hybridised with IGF II cDNA, and then stripped of bound probe and re-hybridised with JIP-1 cDNA. The Northern blots are shown in Figure 1A (IGF II) and Figure 1B (JIP-1). It was found that the expression levels of both genes differed considerably between the fourteen tumours. However, visual examination suggested that there might be a certain amount of co-variation between the expression levels of the two genes. To obtain a more accurate comparison, the intensity of the relevant bands was examined after both hybridisations by densitometry and the integrated absorption values divided by each other. All figures were normalised by multiplication with a constant, so that the relationship in a reference murine embryo (17) was given the relative value 1 (18). It was found that in two tumours only (one embryonal carcinoma and one mixed seminoma-embryonal carcinoma) was the IGF II expression significantly greater than the JIP-1 expression. In the remaining twelve tumours, the relative values were all in the range 0.8-1.3, which indicates a closely controlled relationship between the transcriptional patterns of the two genes.

Discussion

Any attempt to understand the mechanisms that control receptor and cell specificity of MAP kinases will probably help to elucidate the principles of intracellular transduction of growh stimulatory messages. The MAPK pathway anchors individual phosphorylating enzymes to a protein scaffold, which controls the timing and sequence of events and, hence, adds to the specificity of the pathway. The JIP-1 protein, which is a pivotal scaffold protein, was originally isolated by means of its ability to bind JNK proteins (11). Quite unexpectedly, the expression of JIP-1 differed between different tissues in the adult mouse (11), which suggests that the JIP-1 protein plays some important growth regulatory role rather than being a pure housekeeping factor. This was partly followed up by the demonstration of the presence of JIP-1 transcripts in two cell embryos and blastocysts (19). We recently showed that the tissue-related differences observed in adult mice are less augmented in the mouse embryo. Even though there were quantitative differences in JIP-1 expression, each examined organ showed at least a minimum transcriptional activity (18). We also examined whether these observations reflect differences in growth signalling. Since growth regulatory messages are often mediated by polypeptide growth factors, we examined whether the JIP-1 gene expression is in any way related to the activation of a key growth factor. For this type of analysis, IGF II was chosen because of its high rate of transcription in the normal mouse embryo where the JIP-1 gene is also expressed (18).

Since human foetal tissues are limited in supply, human embryonic tumour samples have occasionally been used as a means of indirect analysis of embryonic processes (20). IGF II expression in human testicular tumours has previously been under study and was found to vary according to histological type (1) This is in contrast to carcinoma *in situ* of the testes where IGF II could not be detected (21). Some tumours then displayed a clear over-expression of IGF II. Others, in contrast, produced an unusually low level of IGF II transcripts. Differences in IGF II expression can, however, partly be explained in terms of parental imprinting (22, 23) of the IGF II gene. The reciprocal imprinting of the IGF II and one of its receptor genes has, for example, been used to explain crucial evolutionary equilibria (24). However, parental imprinting of the IGF II gene is not completely stringent even in the human embryo, since in some tissues (as *e.g.* the leptomeninges) transcription progresses in an bi-allelic fashion (25-27).

We found that the expression of IGF II varied considerably between different tumours, thereby confirming earlier reports (1, 3). We can also confirm previous observations that there is a co-variation between IGF II and JIP-1 expression. This study showed that twelve out of fourteen surgically removed testicular tumours display a

co-variation in expression levels of the two genes. This suggests that the expression levels of IGF II and JIP-1 can also be linked under relaxed growth control conditions. It, however, remains to be shown how other members of the MAPK cascade are affected by interference with the embryonic IGF II expression. This work is currently in progress.

Acknowledgements

This study was supported by Cancerfonden, Barncancerfonden, the Swedish Medical Research Council and the Cancer Research Campaign of Great Britain. W.E. was a recipient of an UICC technology transfer fellowship. The authors acknowledge Dr R.J. Davis for the gift of the JIP-1 cDNA probe and Dr Arman Shokrai for the gift of the IGF II cDNA probe.

References

1 Engström W, Hopkins B and Schofield P: Expression of growth regulatory genes in primary human testicular neoplasms. Int J Androl *10*: 79-84, 1987.

2 Schofield PN and Engström W: Insulin like growth factors in human cancer. *In*: The Insulin like Growth Factors - Structure and Biological Function. Schofield PN, ed. Oxford University Press, pp. 240-257, 1990.

3 Engström W, Tally M, Granerus M, Hedley EP and Schofield P: Growth factors and the control of human teratoma cell proliferation. Recent Res Cancer Res *123*: 145-153, 1991.

4 Derijard B, Hibi M, Wu IH, Barrett T, Han J, Ulevitch RJ and Davis RJ: JNK 1 - a protein kinase stimulated by UV light and Ha-Ras that binds and phosphorylates the c-jun activation domain. Cell *76*: 1025-1037, 1994.

5 Garrington TP and Johnsson GL: Organisation and regulation of mitogen activated protein kinase signalling pathways. Curr Op Cell Biol *19*: 211-218, 1999.

6 Sluss HK, Han Z, Barrett T, Davis RJ and Ip T: A JNK signal transduction pathway that mediates morphogenesis and an immune response in *Drosophila*. Genes Develop *10*: 2745-2757, 1996.

7 Xu X, Heidenreich O, Kitajima I, McGuire K, Li Q, Su B and Nerenberg M: Constitutively activated JNK is associated with HTLV-1 mediated tumorigenesis. Oncogene *13*: 135-142, 1996.

8 Shim J, Lee H, Park J, Kim H and Choi EJ: A non enzymatic p21 protein inhibitor of stress activated protein kinases. Nature *381*: 804-807, 1996.

9 Verheij M, Bose R, Hua LX, Yao B, Jarvis W, Grant S, Birrer MJ, Szabo E, Zon LI, Kyriakis JM, Haimovitz-Friedman A, Fuks Z and Kolesnick RN: Requirement for ceramide initiated SAPK/JNK signalling in stress induced apoptosis. Nature *380*: 75-78, 1996.

10 Park J, Kim I, Oh YJ, Lee KW, Han PL and Choi EJ: Activation of c-jun N-terminal kinase antagonises an anti-apoptotic action of Bcl-2. J Biol Chem *272*: 16725-16728, 1997.

11 Dickens M, Rogers JS, Cavanagh J, Raitano A, Xia Z, Halpern JR, Greenberg ME, Sawyers CL and Davis RJ: A cytoplasmic inhibitor of the JNK signal transduction pathway. Science *277*: 693-696, 1997.

12 Whitmarsh AJ, Cavanagh C, Yasuda J and Davis RJ: A mammalian scaffold complex that selectively mediates MAP-kinase activation. Science *281*: 1671-1674, 1998.

13 Verhey KJ, Meyer D, Deehan R, Blenis J, Schnapp BJ, Rapoport TA and Margolis B: Cargo of kinesin identified as JIP scaffolding proteins and associated molecules. J Cell Biol *152*: 959-970, 2001.

14 Barr RK, Kendrick TS and Bogoyevitch MA: Identification of the critical features of a small peptide inhibitor of JNK activity. J Biol Chem *277*: 10987-10997, 2002.

15 Finn SG, Dickens M and Fuller SJ: c-Jun N-terminal kinase interacting protein 1 inhibits gene expression in response to hypertrophic agonists in neonatal rat ventricular myocytes. Biochem J *358*: 489-495, 2001.

16 Hyldahl L, Engström W and Schofield PN: Stimulatory effects of basic fibroblast growth factor on the human embryonic cornea. Development *109*: 693-703, 1990.

17 Engström W, Granerus M and Sachsenmaier W: Differential gene expresion of 3- hydroxy 3-methylglutaryl coenzyme A reductase and N-Myc during the synchronous nuclear cycle of *Physarum polycephalum*. Cell Biol Int Rep *16*: 1133-1137, 1992.

18 Rohbe L, Larsson M, Rising A, Grip S, Burns J and Engström W: Expression of JNK interacting protein JIP-1 is down-regulated in liver from mouse embryos with a disrupted insulin like growth factor II gene. In Vivo *18*: 643-648, 2004.

19 Thompson NA, Haeflinger JA, Senn A, Tawadros T, Magara F, Ledermann B, Nicod P and Waeber G: Islet-brain 1/JNK interacting protein 1 is required for early embryogenesis in mice. J Biol Chem *276*: 27745-27748, 2001.

20 Damjanov I: Pathobiology of human germ cell neoplasia. Rec Res Cancer Res *123*: 1-19: 1991.

21 Drescher B, Lauke H, Hartmann M, Davidoff MS and Zumkeller W: Immunohistochemical pattern of insulin-like growth factor (IGF) I, IGF II and IGF binding proteins 1 to 6 in carcinoma *in situ* of the testis. Mol Pathol *50*: 298-303, 1997.

22 de Chiara T, Robertson E and Efstradiatis A: A growth deficiency phenotype in heterozygous mice carrying an insulin like growth factor II gene disrupted by targetting. Nature *345*: 78-80, 1990.

23 deChiara T, Robertson E and Efstradiatis A: Parental imprinting of the mouse insulin like growth factor II gene. Cell *64*: 849-859, 1991.

24 Haig D and Graham CF: Genomic imprinting and the strange case of the insulin like growth factor II receptor. Cell *64*: 1045-1046, 1991.

25 Ward A, Bierke P, Pettersson E and Engström W: Insulin like growth factors; growth, transgenes and imprinting. Zool Sci *11*: 167-174, 1994.

26 Engström W, Shokrai A, Otte K, Granerus M, Gessbo , Bierke P, Madej A, Sjölund M and Ward A: Transcriptional regulation and biological significance of the insulin like growth factor II gene. Cell Prol *31*: 1-16, 1998.

27 Dell G, Ward A, Shokrai A, Madej A and Engström W: Regulation of the IGF system by Glucocorticoids. Zool Sci *16*: 377-385, 1999.

Received October 5, 2004
Revised February 7, 2005
Accepted February 18, 2005